Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

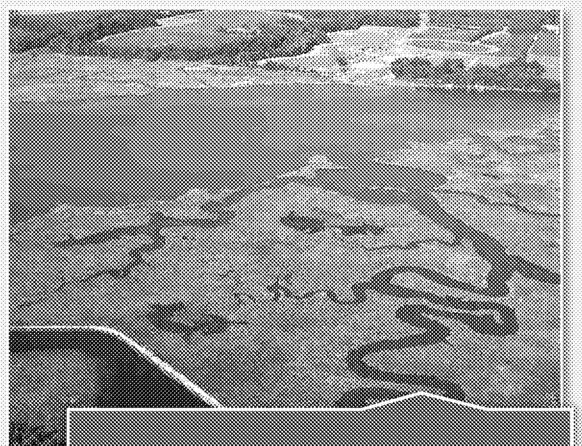
National Priorities: Per- and Polyfluoroalkyl Substances Grant Kickoff Meeting

September 5, 2019
Durham, NC

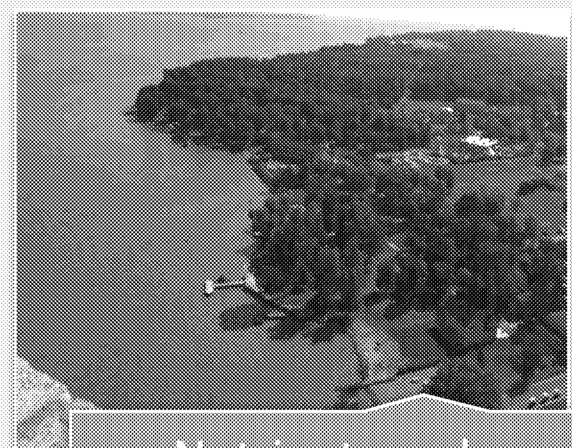
Overarching Research Topics

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

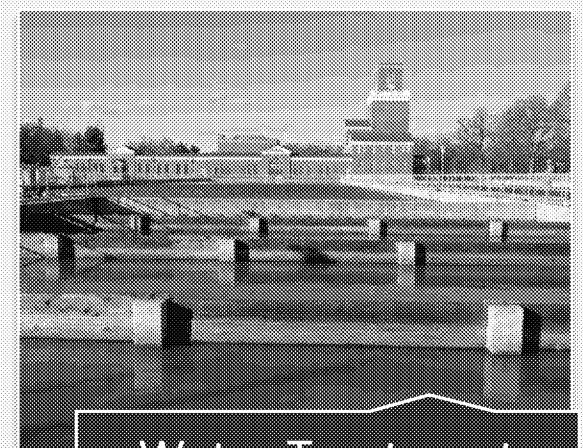
Clean Water Act | Safe Drinking Water Act



Watersheds



Nutrients and Harmful Algal Blooms



Water Treatment and Infrastructure

RFA Research Areas

Research that will advance current knowledge of PFAS fate and transport, human and ecological exposure, and toxicity. Proposed approaches, tools, and data should contribute to enabling states, tribes, and local communities to make informed decisions about the assessment, management, and communication of risk from PFAS contamination in water.

PFAS fate and transport

- *Source areas*
- *Movement*
- *Transport properties*
- *Transformations and degradations*

PFAS human and ecological exposure

- *Occurrence, identity, Sources, and concentrations*
- *Human activity patterns*
- *Relative source contribution*
- *Chemical mixtures and individual chemicals*

PFAS Toxicity

- *Investigate adverse health, aquatic, and terrestrial ecological effects*
- *Cellular and molecular mechanisms of toxicity*
- *Biomonitoring or epidemiological studies*
- *Risk assessment methods*

Colorado School of Mines

Christopher Higgins, John Adgate, Courtney Carignan, Jane Hoppin, Tissa Illangasekare, Detlef Knappe, Heather Stapleton

PFAS UNITEDD: Poly- and Perfluoroalkyl Substances-U.S. National Investigation of Transport & Exposure from Drinking Water and Diet

chiggins@mines.edu

EPA

Oregon State University

Robert Tanguay, Jamie DeWitt, Jennifer Field, Carla Ng, David Reif, and Lisa Truong

System toxicological approaches to define and predict the toxicity of Per and Polyfluoroalkyl Substances

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http://tanguaylab.com/

Sinnhuber Aquatic Research Laboratory

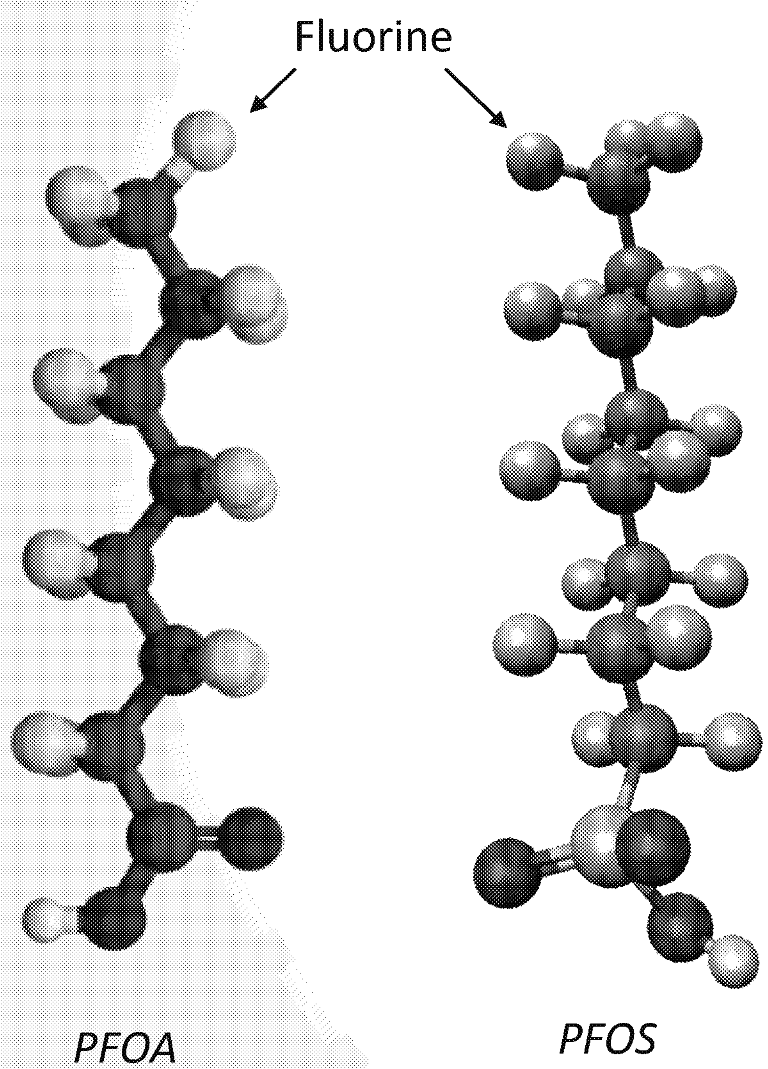
Agenda

9:00 am:	Welcome and Introductions – Ben Packard
9:10 am:	EPA PFAS Research overview – Andy Gillespie
9:25 am:	Colorado School of Mines - Chris Higgins, Detlef Knappe, and Courtney Carignan
10:25 am:	Break
10:40 am:	High Resolution Mass Spectrometry (HRMS) investigations of PFAS – Mark Strynar
11:10 am:	Discussion – Potential collaboration
12:00 pm:	Lunch
12:30 pm:	Oregon State University - Robert Tanguay, Jennifer Field, Jamie Dewitt, Carla Ng and David Reif
1:30 pm:	Office of Research and Development Tiered Testing Research Overview – Stephanie Padilla
2:00 pm:	Discussion – Potential Collaboration
3:00 pm:	Adjourn

US EPA's Science-Based Approach to Understanding and Managing Environmental Risk from PFAS

Andrew J. R. Gillespie, Ph. D.
Associate Director, National Exposure Research Laboratory
Executive Lead for PFAS Research and Development
US Environmental Protection Agency

Per- & Polyfluoroalkyl Substances (PFAS)



- **A class of man-made chemicals**
  - **Chains** of carbon (C) atoms surrounded by fluorine (F) atoms, with different terminal ends
  - **Complicated chemistry** – thousands of different variations exist in commerce
  - **Widely used** in industrial processes and in consumer products
  - **Some** PFAS are known to be **PBT**:
    - **Persistent** in the environment
    - **Bioaccumulative** in organisms
    - **Toxic** at relatively low (ppt) levels

Recent EPA Actions on PFAS

- National PFAS Leadership Summit - May 2018
  - Share information, identify actions, risk communication
- Near Term EPA Actions Announced at Summit
  - Develop groundwater cleanup recommendations for PFOA/PFOS (OLEM)
  - Examine options for listing PFOA/PFOS as Hazardous Substances (OLEM)
  - Release toxicity assessments for GenX and PFBS by fall (OW & ORD)
- Community Events June-Sept 2018
  - Series of 6 public meetings on PFAS concerns
- EPA PFAS Action Plan - February 14 2019
  - Building on lessons learned from Summit, Engagements, Docket

EPA PFAS Action Plan

- Provides EPA's first multi-media, multi-program, national research, management and risk communication plan to address a challenge like PFAS.
- Responds to the extensive public input the agency has received over the past year during the PFAS National Leadership Summit, multiple community engagements, and through the public docket.
- As a result of this unprecedented outreach, the Action Plan provides the necessary tools to assist states, tribes, and communities in addressing PFAS.

EPA PFAS Action Plan

- **Drinking Water** – The EPA is committed to following the MCL rulemaking process as established by SDWA. EPA will propose a regulatory determination for PFOA and PFOS by the end of this year, and propose nationwide drinking water monitoring for PFAS under the next UCMR monitoring cycle.
- **Cleanup** – Initiating the regulatory process for designating PFOA and PFOS as Hazardous Substances, set interim groundwater cleanup recommendation
- **Toxics** – Consider including PFAS in Toxics Release Inventory (TRI), initiate proposal to prohibit the uses of certain PFAS chemicals through the TSCA new chemicals program
- **Research** – Rapidly expand scientific foundation for understanding and managing PFAS risk
- **Enforcement** – Use enforcement tools, where appropriate, to address PFAS exposures in the environment and assist states in enforcement activities
- **Risk Communications** – Work with partners to develop a risk communication toolbox to support federal, state, tribal, and local partners for communicating with their constituents

PFAS Action Plan - Research

- The EPA is rapidly expanding the scientific foundation for understanding and managing risk from PFAS.
- This research is organized around:
  - understanding **toxicity**
  - understanding **exposure**
  - assessing **risk**
  - identifying effective **treatment and remediation** actions

Research – Human Health

- **Problem**: Lack of human toxicity information for many PFAS of interest
- **Action**: 2-prong strategy
  - Develop standard toxicity assessments (e.g. IRIS) where data are available
  - Use in vitro, high throughput screening approaches to fill in gaps
- **Results**:
  - Initial search of published toxicity data for 31 PFAS of interest, ~21 have data
  - Draft toxicity assessments available for HFPO-DA (GenX) and PFBS
  - Draft IRIS assessments underway for PFBA, PFHxS, PFHxA, PFNA and PFDA
  - Seven sets of high throughput assays underway for 150 PFAS representative of chemical space to support prioritization for further tox testing, chemical grouping, read across, relative toxicity and mixtures assessment
- **Impact**: Stakeholders will have PFAS toxicity information to inform risk management decisions and risk communication

Research – Ecological Toxicity

- **Problem**: Lack of ecological toxicity information for PFAS of concern
- **Action**:
  - Systematic review of literature, assembled in the ECOTOX database
  - Developing research plan including identification of sensitive taxa, bioaccumulation, benchmarks, and thresholds
  - Use Adverse Outcome Pathways (AOP) as organizational framework
- **Results**:
  - 374 references, ~83 PFAS, 189 species curated in ECOTOX knowledgebase
  - AOP development, verification getting underway
- **Impact**: Stakeholders will have PFAS ecotoxicity information to support risk management decisions and risk communication

Research – Analytical Methods

- **Problem**: Lack of standardized/validated analytical methods for measuring PFAS
- **Action**: Develop and validate analytical methods for detecting, quantifying PFAS in water, air, and solids
- **Results**:
  - Validated analytical Method 537.1 for **drinking water** which includes 4 additional PFAS (18 total, including HFPO-DA and ADONA)
  - Developing new **DW** Method for ~26 PFAS including shorter chains
  - Validating Direct Injection and Isotope Dilution methods for 24 PFAS in **surface water, ground water, soils, sediments, and biosolids**
  - Developing methods for **air emission** sampling and analysis
  - Continued development of **HR mass spec methods** to discover unknown PFAS
- **Impact**: Stakeholders will have reliable analytical methods to test for known and new PFAS in water, solids, and air

Research – Exposure

- **Problem**: Lack of knowledge on sources, site-specific concentrations, fate and transport, bioaccumulation, and human and ecological exposure
- **Action**: Develop and test methods, models, and databases to characterize PFAS sources and exposures
- **Results**:
  - Developing exposure models for identifying, quantifying PFAS sources, fate and transport pathways, and exposures
  - Developing and evaluating sampling and site characterization approaches to identify sources and extent of contamination
- **Impact**: Stakeholders will be able to identify and assess potential PFAS sources and exposures, and identify key pathways for risk management

Research – Drinking Water Treatment

- **Problem**: Lack of water treatment technology performance and cost data for PFAS removal
- **Action**:
  - Review PFAS performance data from available sources (industry, DoD, academia, international)
  - Test commercially available granular activated carbons (GACs) and ion exchange (IX) resins for effectiveness over a range of PFAS under different water quality conditions
  - Evaluate a range of system sizes – large full-scale utility options to home treatment systems
- **Results**:
  - EPA's **Drinking Water Treatability Database** updated for 22 PFAS, including HFPO-DA (GenX chemicals), 6:2 and 8:2 FTS, and PFAS of 4 to 13 C chain length
  - Use state-of-the-science models to extrapolate existing treatment studies to other conditions
- **Impact**: Utilities will be able to identify cost effective treatment strategies for removing PFAS from drinking water

Research – Contaminated Site Remediation

- **Problem**: PFAS-contaminated sites require remediation and clean up to protect human health and the environment
- **Action**:
  - Characterize PFAS sources such as fire training/emergency response sites, manufacturing facilities, production facilities, disposal sites
  - Evaluate technologies for remediating PFAS-impacted soils, waters, and sediments
  - Generate performance and cost data with collaborators to develop models and provide tools to determine optimal treatment choices
- **Results**: Tools, data and guidance regarding cost, efficacy, and implementation for remedy selection and performance monitoring
- **Impact**: Responsible officials will know how to reduce risk of PFAS exposure and effects at contaminated sites, and to repurpose sites for beneficial use

Research – Materials Management

- **Problem**: Lack of knowledge regarding end-of-life management of PFAS-containing consumer and industrial products
- **Action:**
  - Characterize end-of-life PFAS disposal streams (e.g. municipal, industrial, manufacturing, recycled waste streams)
  - Evaluate efficacy of materials management technologies (e.g. landfilling, incineration, composting, stabilization) to manage end-of-life disposal
  - Evaluate performance and cost data with collaborators to manage these materials and avoid environmental PFAS releases
- **Results**: Provide technologies, data and tools to manage end-of-life streams
- **Impact**: Responsible officials will be able to manage effectively end-of-life disposal of PFAS-containing products

Technical Assistance

- **Problem**: State, tribes and communities often lack capabilities for managing PFAS risk
- **Action:**
  - Make EPA technical staff available to consult on PFAS issues
  - Utilize applied research while also providing technical support to site managers
  - Summarize and share lessons learned from technical support activities
- **Results**: Many examples of past and ongoing technical assistance
  - **Cape Fear River, NC** – Significant reductions in PFAS in source and finished water
  - **Manchester, NH** – Collaboration on air and water sampling
  - **Oscoda, MI** – Advice on foam sampling and dermal exposure risk on a recreational lake
- **Impact**: Enable states, tribes and communities to 'take action on PFAS'

Collaboration

PFAS is a topic of interest to many different organizations, and EPA is committed to leveraging partnerships and collaborations to achieve results. Some examples:

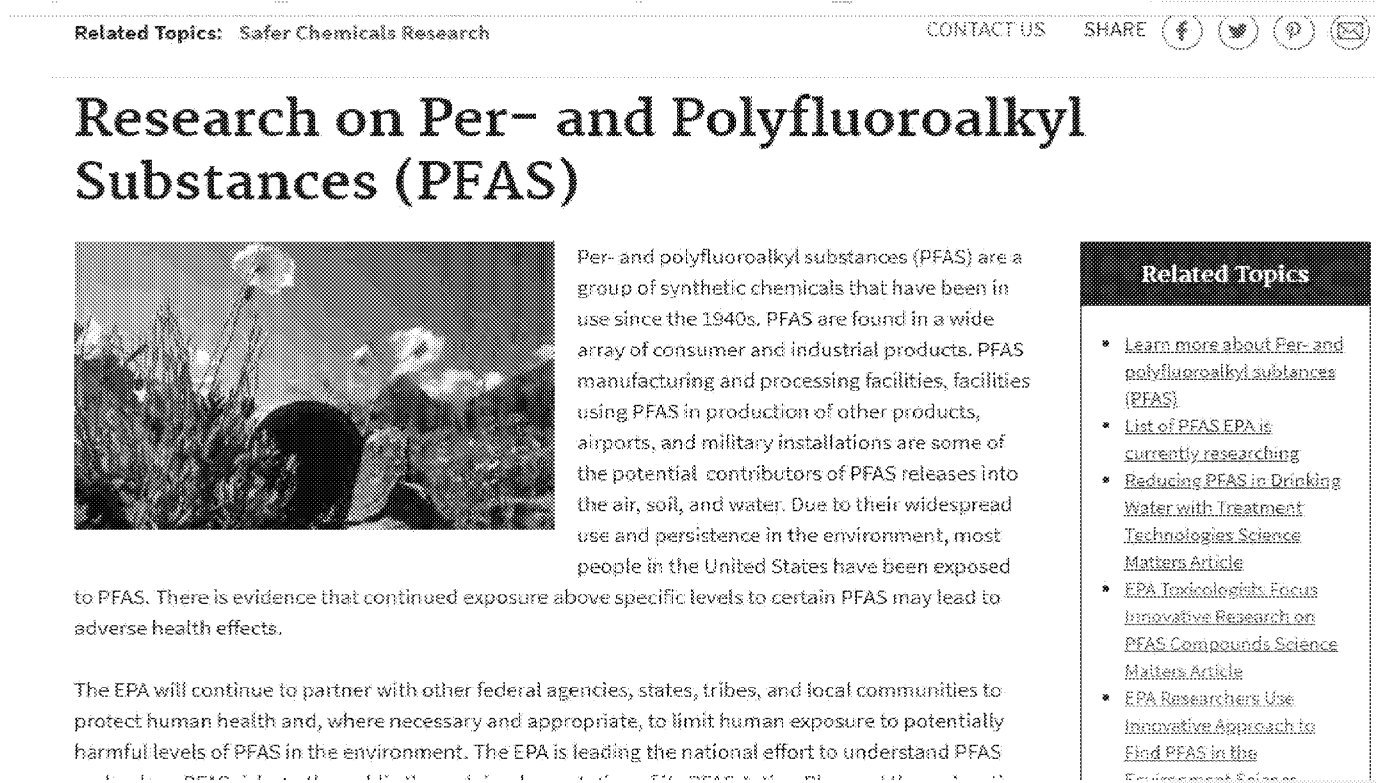
- Collaborating with the **National Toxicology Program** (NTP) on high throughput toxicology testing
- Collaborating with **DOD** on analytical method development, treatment/remediation approaches, and participation in the Strategic Environmental Research and Development Program (SERDP)
- Collaborating with **individual states and public utilities** in testing and applying PFAS measurement and treatment methods
- Collaborating with the **academic community** via EPA's Science to Achieve Results (STAR) competitive grant program

EPA PFAS Data and Tools

- Links to data and tools that include information related to PFAS and are available on EPA's website:

https://www.epa.gov/pfas

https://www.epa.gov/chemical-research/research-and-polyfluoroalkyl-substances-pfas


Related Topics: Safer Chemicals Research CONTACT US SHARE

Research on Per- and Polyfluoroalkyl Substances (PFAS)

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been in use since the 1940s. PFAS are found in a wide array of consumer and industrial products. PFAS manufacturing and processing facilities, facilities using PFAS in production of other products, airports, and military installations are some of the potential contributors of PFAS releases into the air, soil, and water. Due to their widespread use and persistence in the environment, most people in the United States have been exposed to PFAS. There is evidence that continued exposure above specific levels to certain PFAS may lead to adverse health effects.

The EPA will continue to partner with other federal agencies, states, tribes, and local communities to protect human health and, where necessary and appropriate, to limit human exposure to potentially harmful levels of PFAS in the environment. The EPA is leading the national effort to understand PFAS

Related Topics

- Learn more about Per- and polyfluoroalkyl substances (PFAS)
- List of PFAS EPA is currently researching
- Reducing PFAS in Drinking Water with Treatment Technologies Science Matters Article
- EPA Toxicologists Focus Innovative Research on PFAS Compounds Science Matters Article
- EPA Researchers Use Innovative Approach to Find PFAS in the

For More Information

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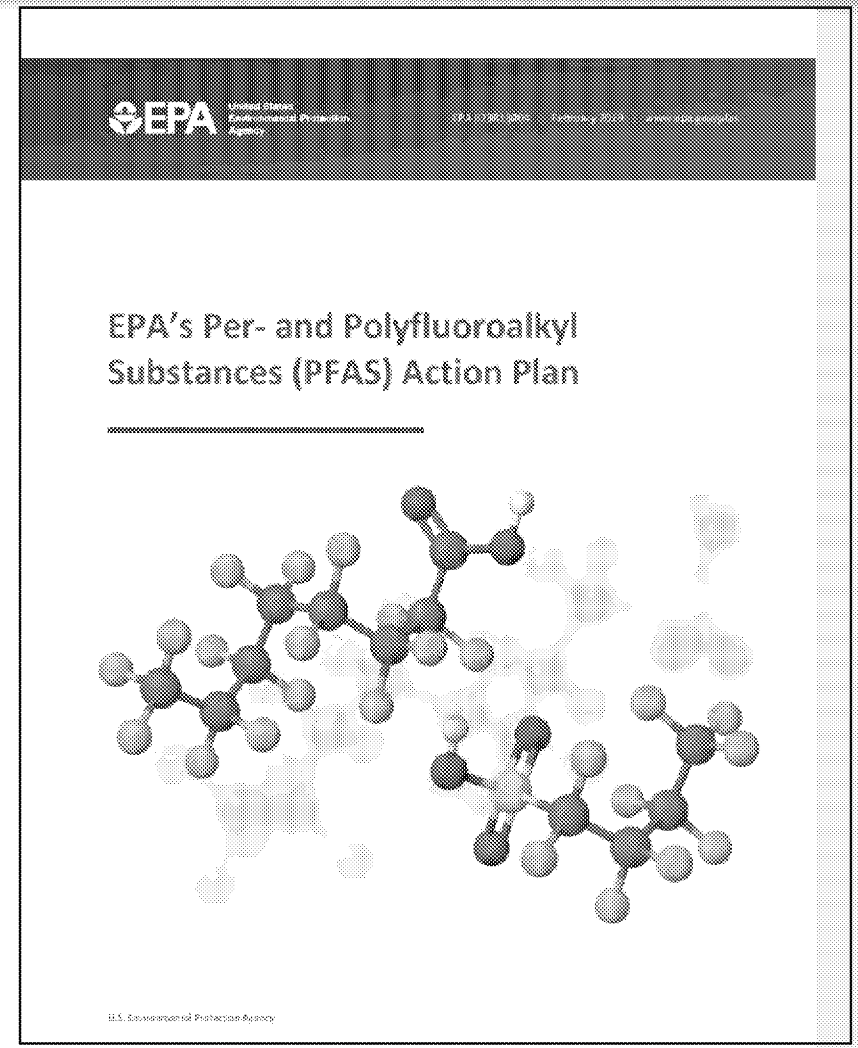


The views expressed in this presentation are those of the individual author and do not necessarily reflect the views and policies of the US EPA

:: PFAS UNITEDD ::

U.S. National Investigation of Transport and Exposure from Drinking Water and Diet

U.S. EPA Grant Number: G18A112656081

www.pfasunitedd.org

Christopher Higgins

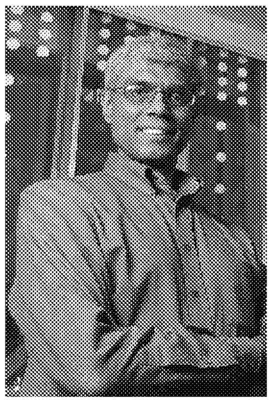
Tissa Illangasekare, Detlef Knappe, Owen Duckworth, Jane Hoppin, Heather Stapleton, Courtney Carignan, and John Adgate

Outline

- Project Team
- Motivating Questions and Overall Objectives
- Project Structure
- Approach
  - Aim 1: Critical Data Gaps on Environmental Transport of Overlooked PFASs
  - Aim 2: Uptake of PFASs into Local Foods
  - Aim 3: Assessing the Relative Roles of Drinking Water, Diet, and Indoor Exposure
- Advisory Board
- Project Schedule

Project Team – Leadership

Christopher Higgins
Colorado School of Mines



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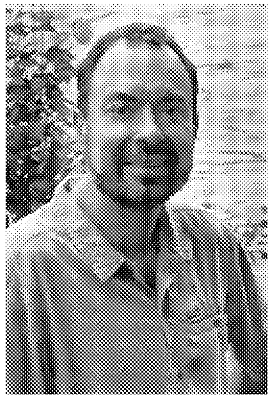

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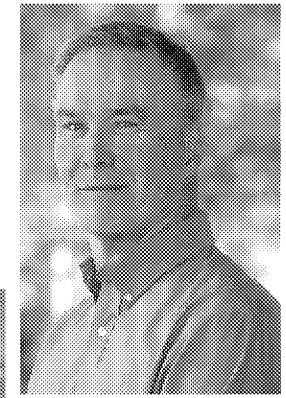
Duke University

Courtney Carignan
Michigan State University



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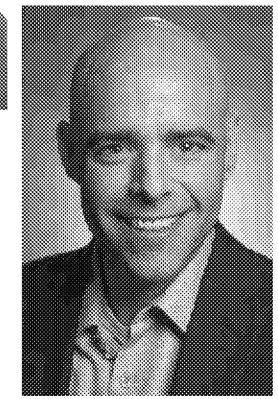
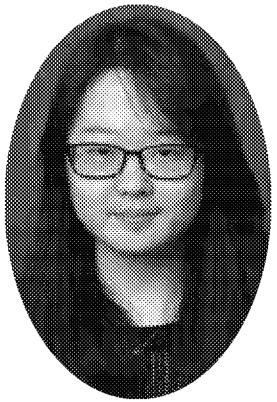
Colorado School of Public Health



Owen Duckworth
North Carolina State University

Sydney Binette
QA Representative, Colorado School of Mines

Project Team – Students, Postdoctoral Fellows



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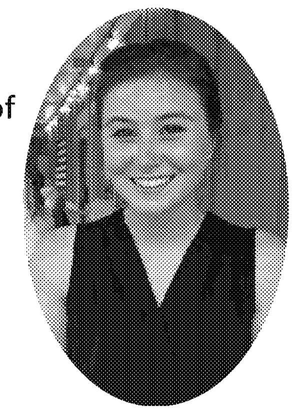

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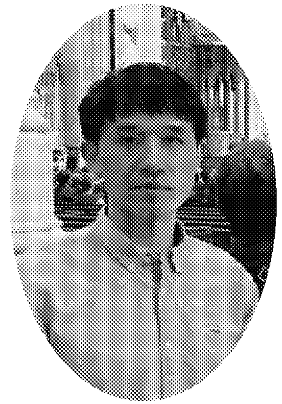

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**Ankita
Bhattacharya**
PhD Student,
Michigan State
University

Project Team - Partners

- Jason Conder, Geosyntec
- Jim Hatton, Jacobs
- Charles Schaefer, CDM Smith
- Robin Vestergren, IVL

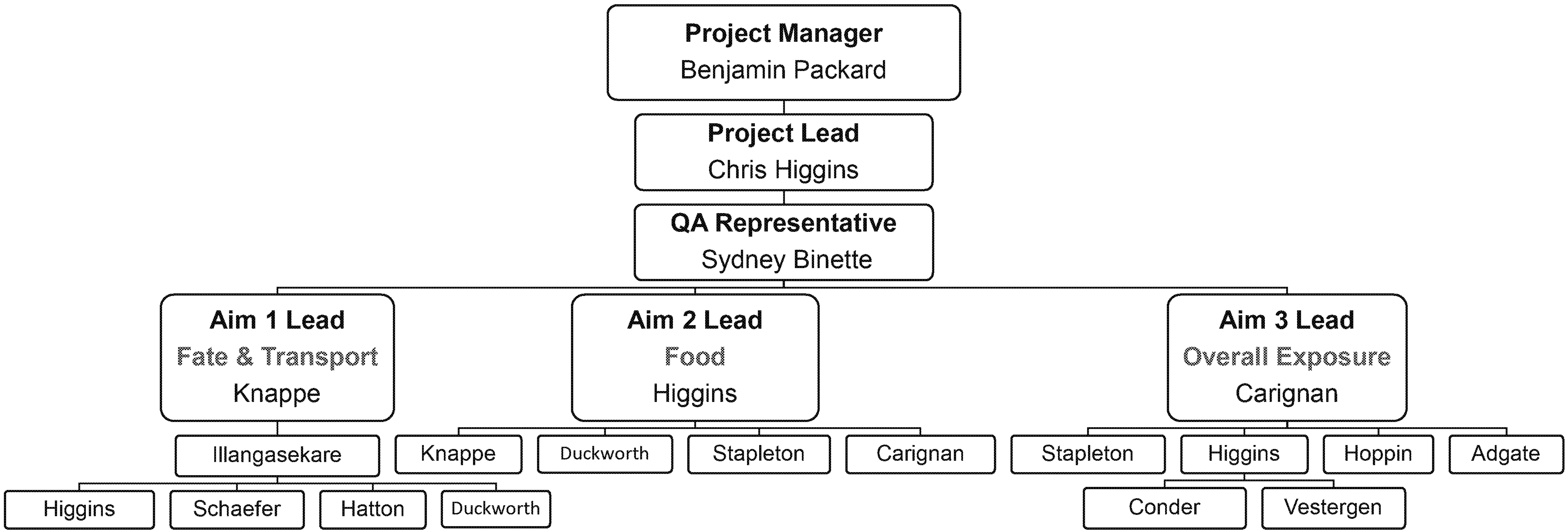
Motivating Questions

- Once a point source of PFASs is identified, *what data are needed* to predict the extent of contamination and how quickly will PFASs migrate, particularly through soil to groundwater?
- Which PFASs and to what extent are PFASs *taken up in locally grown/raised/caught food* in communities impacted by PFAS drinking water contamination?
- *Will treating impacted water be sufficient* to reduce human exposure to PFASs to "background" exposure? That is, if the drinking water is treated but the contaminated water (or soil) continues to be used for crops, gardens, and livestock, will serum levels revert to U.S. background?

Overall Objectives

- Develop *actionable* data on the fate, transport, bioaccumulation, and exposure of a diverse suite of poly- and perfluoroalkyl substances (PFASs) in nationally representative PFAS-impacted communities to enable reductions in total PFAS exposure.
- Collect key data on fate, transport, bioaccumulation, and exposure that are currently lacking for many PFASs. Specific data to be collected include:
  - Desorption/sorption data for some overlooked PFASs
  - Vadose-zone transport data for overlooked PFASs
  - Plant uptake data of overlooked PFASs
  - PFAS levels in local food in PFAS-impacted vs. non-impacted areas (MI, NC)
  - PFAS levels in indoor environments in PFAS-impacted vs. non-impacted areas (MI)
  - Relative contributions of drinking water vs. food vs. indoor environment in PFAS-impacted areas (MI)
- Develop a new biomonitoring cohort (MI) and compare MI biomonitoring data to existing CO and NC cohorts in the context of source fingerprinting

Organization and Project Structure



Approach

- Aim 1: Develop new data for sorption/desorption and vadose-zone transport of overlooked PFASs
- Aim 2: Evaluate which PFASs can bioaccumulate in plants and determine levels of PFASs in local foods (plant-based and animal-based) in two PFAS-impacted communities (MI and NC)
- Aim 3: Conduct a detailed exposure assessment in a new MI biomonitoring cohort and compare those results to findings from PFAS-impacted communities in CO and NC

PFAS-UNITEDD Communities

Cohort	Colorado (CO)	North Carolina (NC)	Michigan (MI)
Study Name	PFAS-AWARE (www.PFAS-AWARE.org)	GenX Exposure Study	PFAS UNITEDD (This Proposal)
Known/Suspected PFAS Source for Drinking Water	Aqueous Film Forming Foam (AFFF)	PFAS Manufacturing	Secondary PFAS Use at Paper Mill
Initial cohort funding source (Lead PI)	NIH – R21 (Adgate)	NIH – R21 (Hoppin)	This Proposal (Carignan)
Cohort Size	220 adults	430 adults 70 children	200 adults 200 children

Expected Outcomes

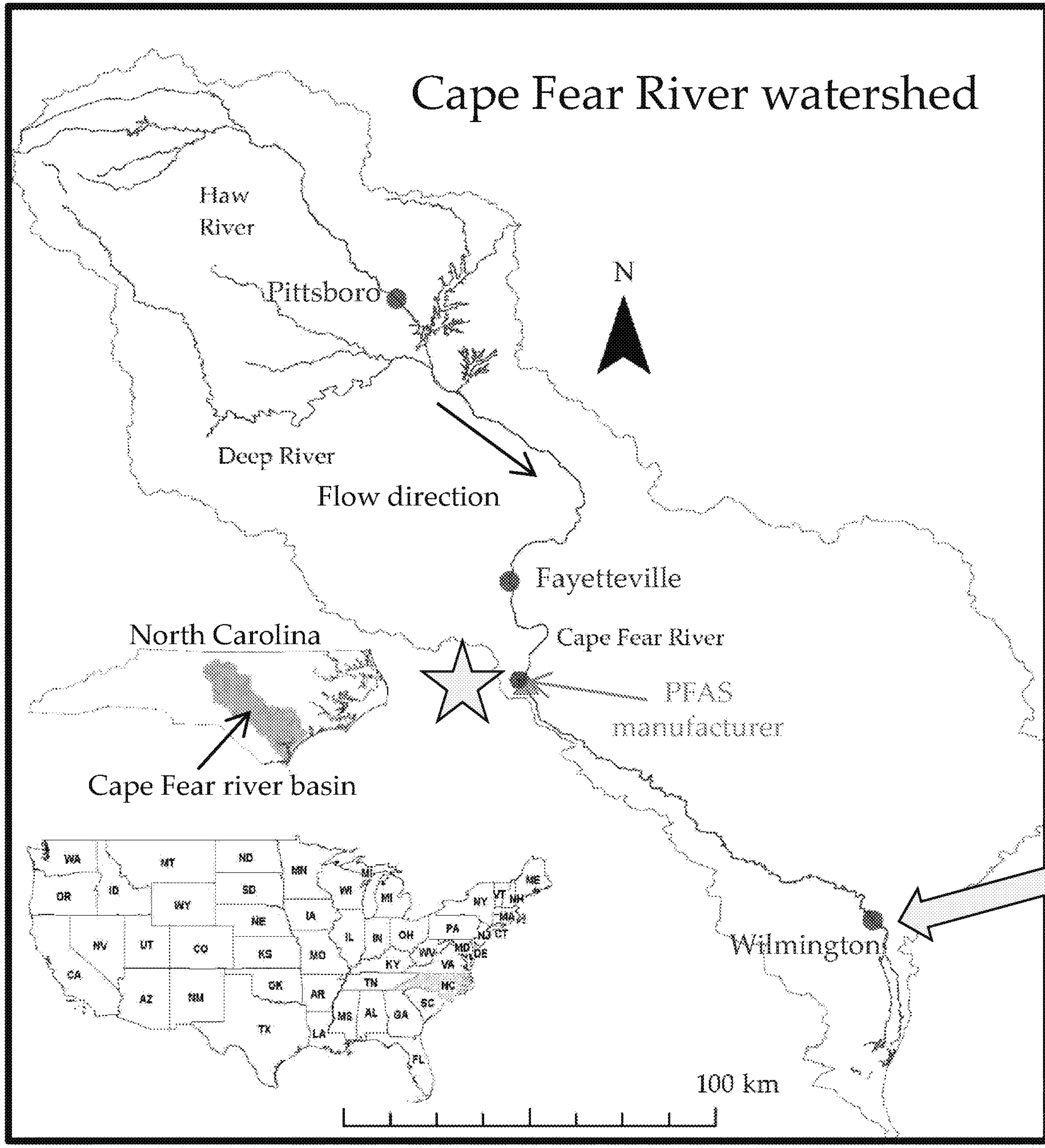
Our goal is to enable state and local risk managers to:

- Quantitatively predict subsurface transport of *all* PFASs;
- Predict accumulation of *all* PFASs in foods from soil or water;
- Better communicate with impacted communities; and
- Understand the importance of different PFAS exposure pathways.

Aim 1: Fill in critical data gaps for the environmental transport of overlooked PFASs

Hypotheses

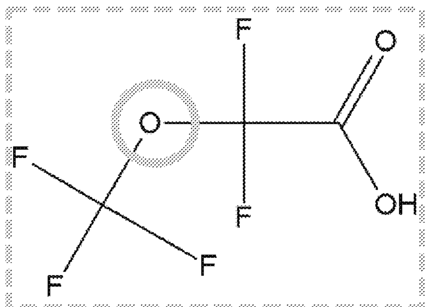
- *H1a: K_{oc} values for PFASs can be predicted from liquid chromatography (LC) retention.*
- *H1b: PFAS sorptive hysteresis becomes more pronounced with increasing solid contact time.*
- *H1c: Vadose zone PFAS transport is dictated by PFAS properties and the degree of saturation.*



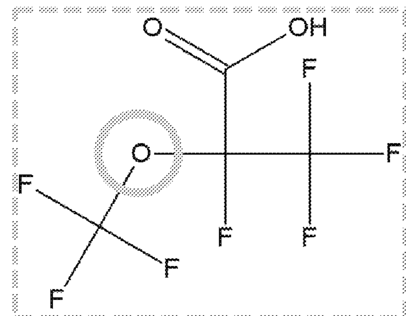
- Largest watershed in NC
- Supplies ~1.5M people with drinking water
- In Wilmington, only **PFHpA (C7)** was detected in UCMR3 samples (concentrations ranged from <10 to 27 ng/L)

Recently identified per- and polyfluoroalkyl ether acids (PFEA) in the Cape Fear River

(1) Mono-ether carboxylic acids with three to six carbon atoms – all perfluorinated

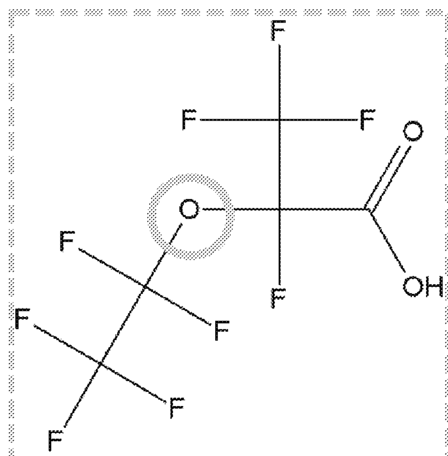


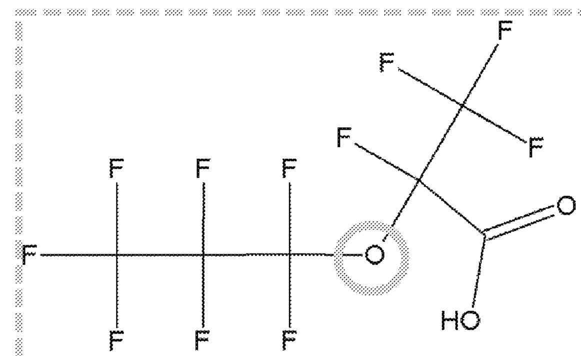
PFMOAA



PMPA

PEPA



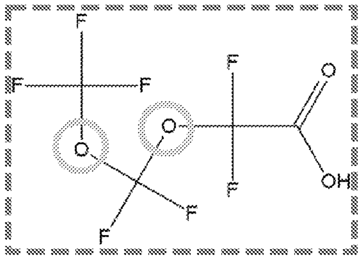


PFPrOPrA (“GenX”)

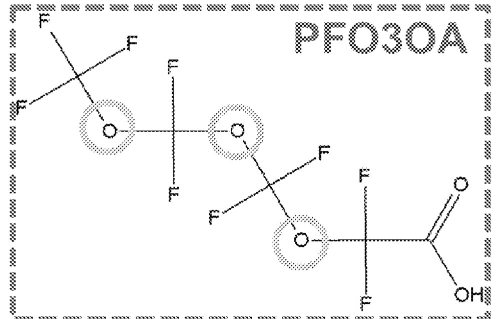
Strynar et al. (2015) ES&T
Hopkins et al. (2018) JAWWA

PFEAs in the Cape Fear River

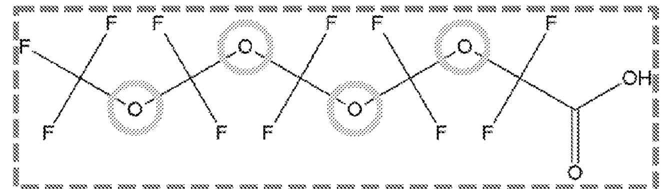
(2) Multi-ether carboxylic acids with up to five ether oxygen atoms (all perfluorinated)



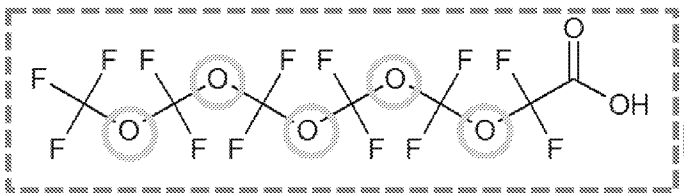
PFO2HxA



PFO3OA



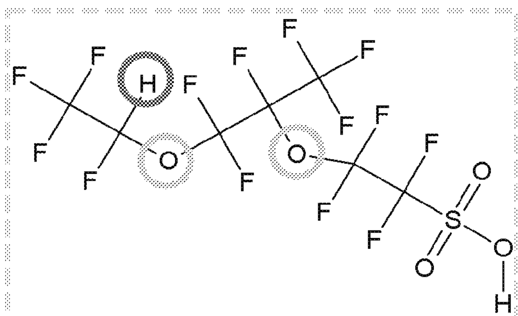
PFO4DA



PFO5DoA

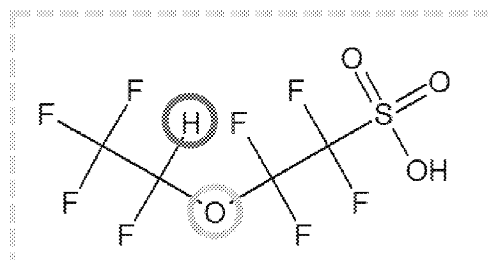
Detected in the blood of Wilmington residents

(3) Polyfluorinated ether acids

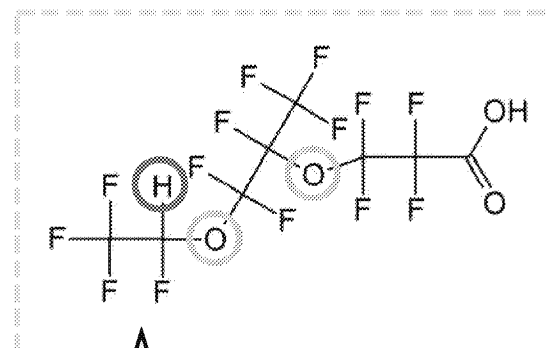


(99%)

Nafion by-product 2

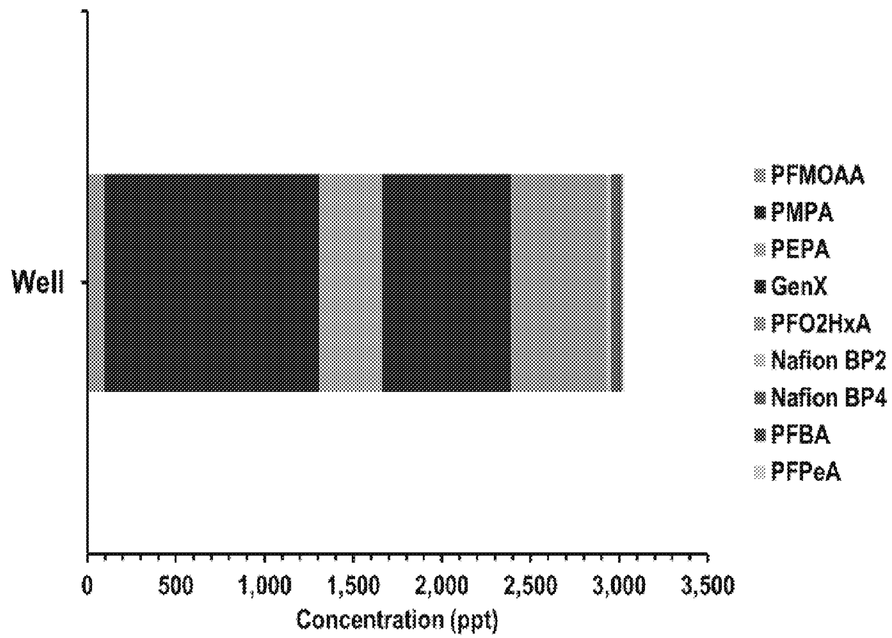


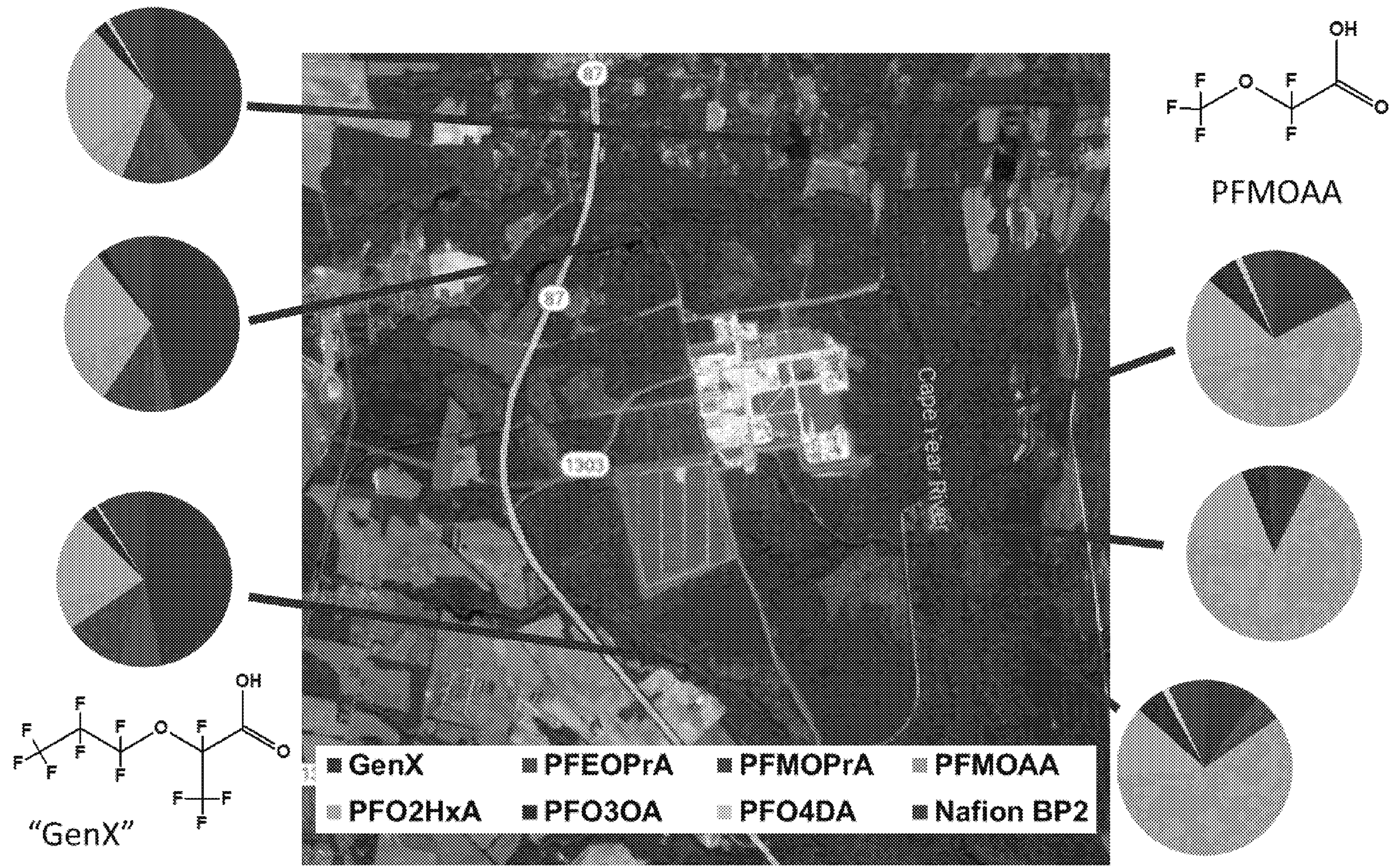
NVHOS



HydroEVE

GenX dominates in water impacted by air emissions, PFMOAA dominates in water impacted by wastewater discharge



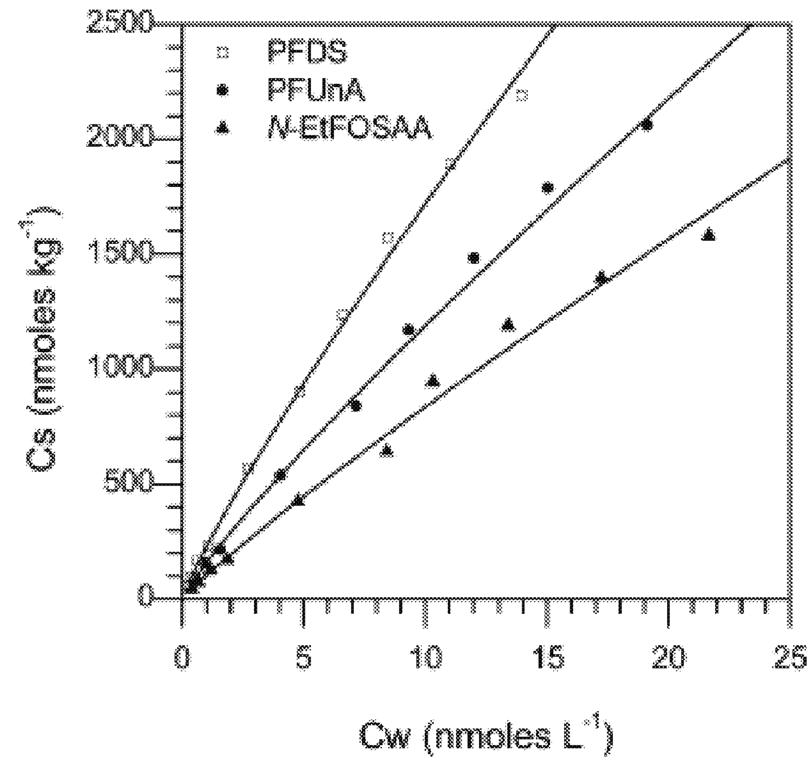


Task 1a. Sorption equilibrium parameters for overlooked PFASs

Goal: Determine K_{oc} values for overlooked PFASs

Approach:

- Batch sorption isotherm experiments
- 5 “clean” NC soils covering a range of textures and organic carbon (OC) contents
- Up to 15 PFEAs in addition to well-studied PFAAs for reference
- Simulated groundwater and impacted groundwater
- Effect of pH
- Single-solute versus multi-solute
- Monovalent cations versus divalent cations



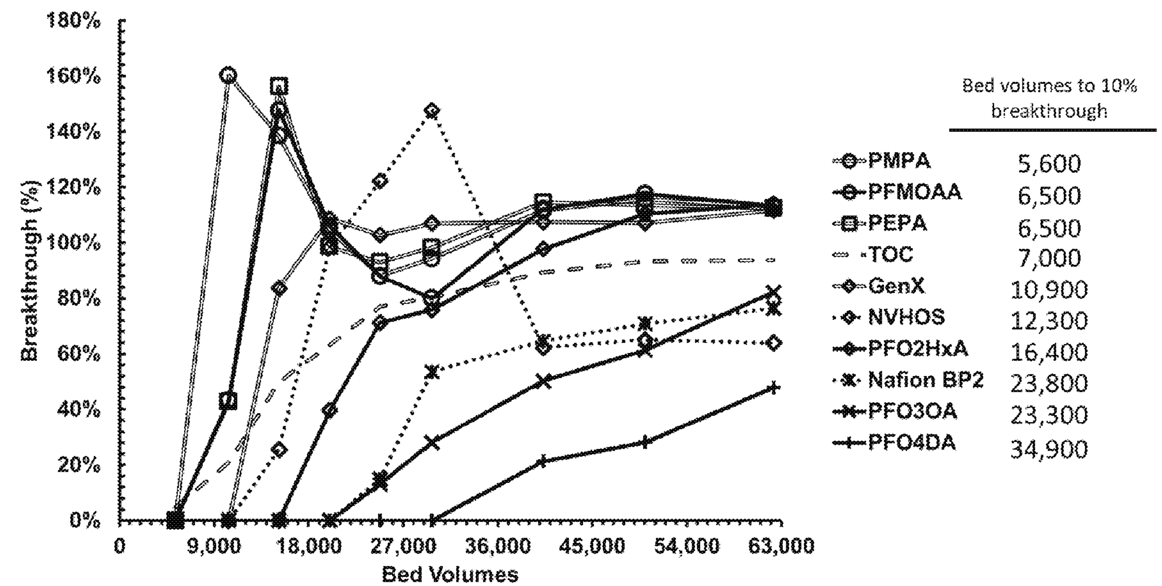
Higgins and Luthy, 2006

Task 1b. Kinetic parameters describing PFAS sorption/desorption

Goal: To characterize sorption/desorption kinetics and establish any effects of aging on PFAS sorption hysteresis

Approach

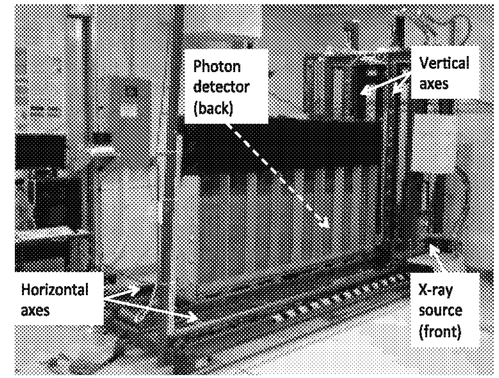
- Batch sorption-desorption experiments
  - Desorption by sequential dilution
  - Assess effect of "aging" on desorption kinetics/equilibrium (hysteresis?)
- Capillary-tube experiments to directly determine diffusion coefficients (Charles Schaefer, CDM-Smith)
- Column experiments to determine retardation factors and sorption/desorption kinetics

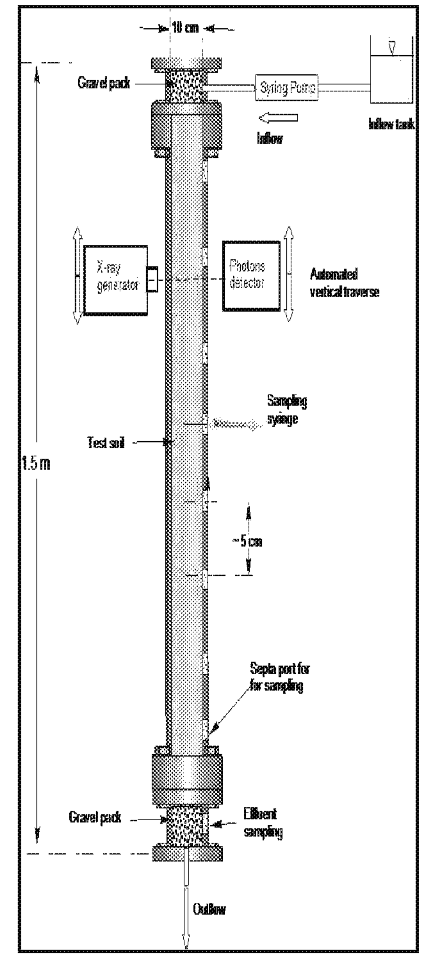


Hopkins et al., in preparation

Task 1c. PFAS Transport under Unsaturated Conditions

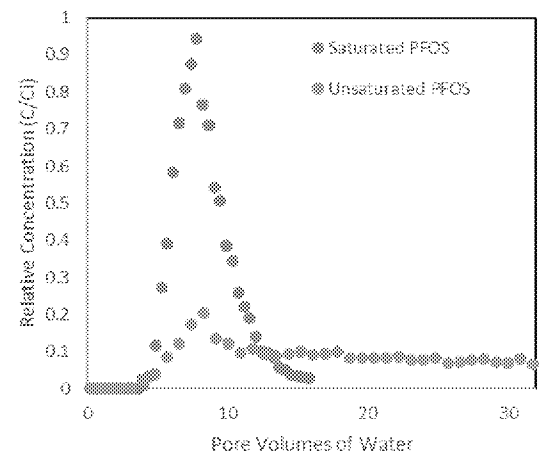
Goal: to characterize the behavior of overlooked PFASs in *unsaturated* soil using hydraulic parameters (soil-water characteristics and permeability)





Laboratory column with traversing x-ray system

- x-ray attenuation estimates and water and air saturations
- long column format creates uniform water saturation
- breakthrough curves for overlooked PFASs (contaminated groundwater)
- controls = clean sands & saturated flow for baseline hydraulic conductivity and dispersivity



Interfacial tension measurements (CDM-Smith)

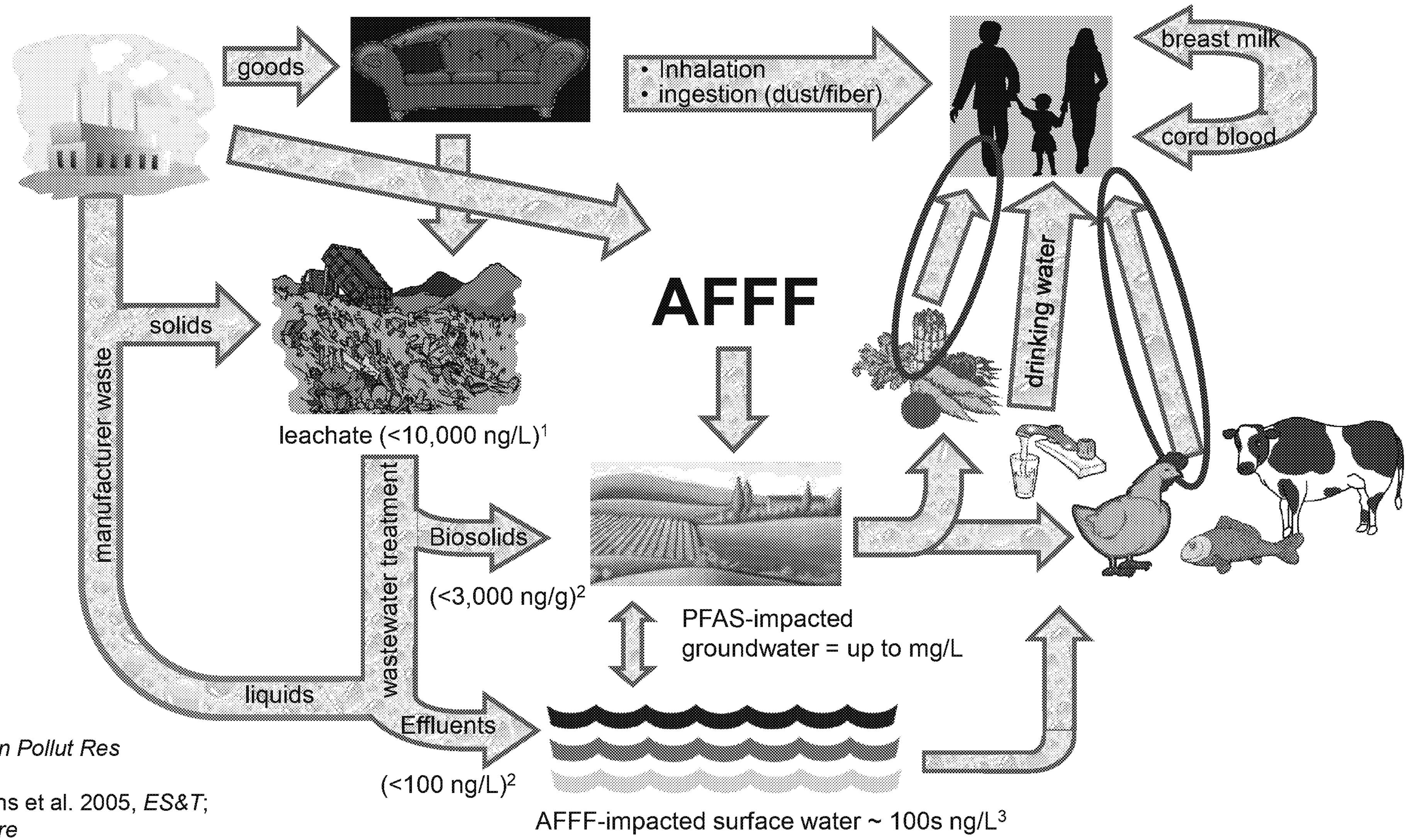
- pendant drop method
- Link surface tension to PFAS concentration and type

Aim 2: Evaluate uptake of PFASs into foods

- *Hypothesis 2a:* ***Uptake of overlooked PFASs into plants can be predicted*** from retention on a liquid chromatography (LC) column

- *Hypothesis 2b:* PFAS levels in locally grown/caught/raised food in PFAS-impacted areas ***will be elevated*** relative to levels in the same foods obtained from non-impacted areas.

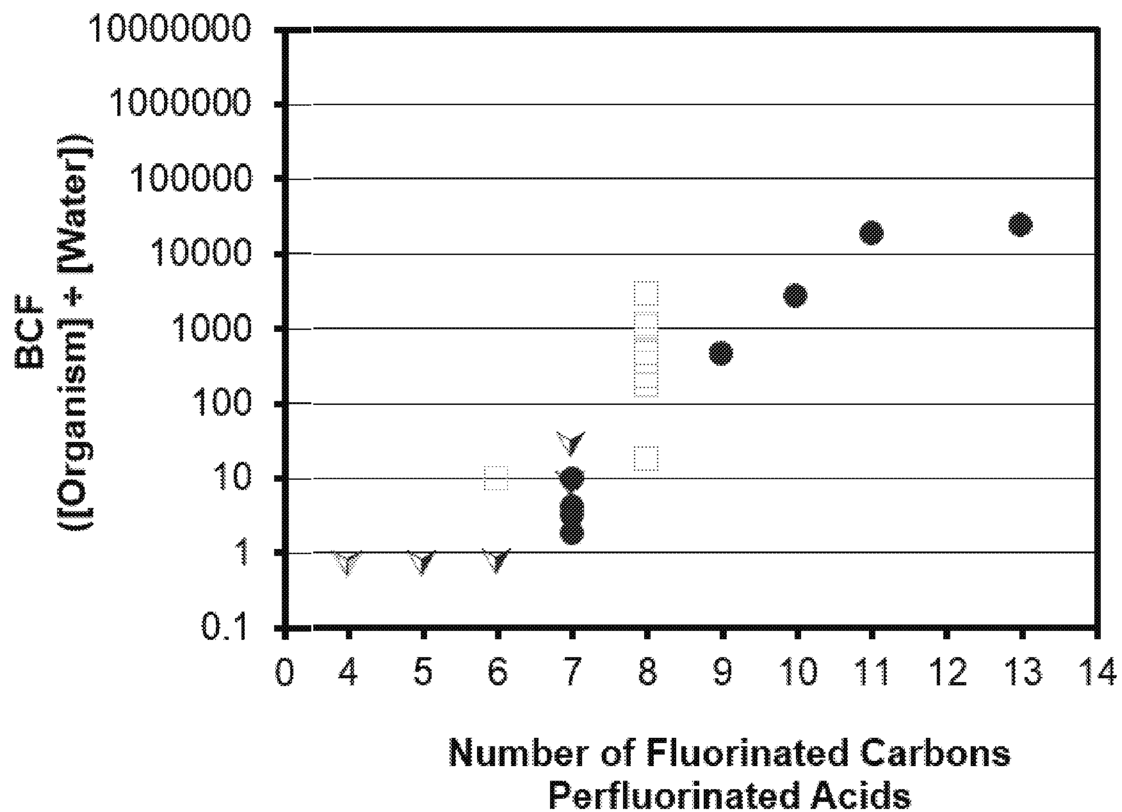
Human Exposure to PFASs



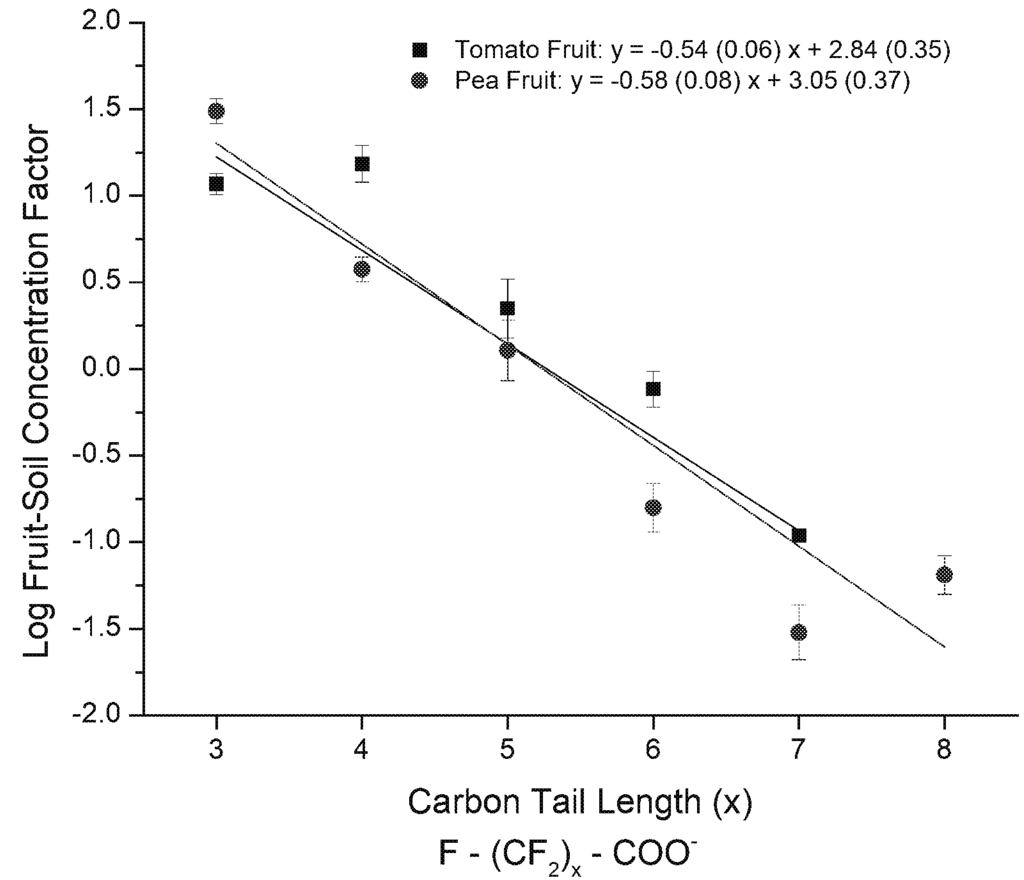
Adapted from Oliaei 2013, *Environ Pollut Res*
[1]Allred *et al.*, 2014. *J Chrom*;
[2]Schultz *et al.* 2006, *ES&T*; Higgins et al. 2005, *ES&T*;
[3]Ahrens *et al.* 2015, *Chemosphere*

Slide content courtesy of ESTCP Project ER-201574-T2. Full FAQ presentation available at https://youtu.be/lyzSoEF792E

PFAS Accumulation Patterns



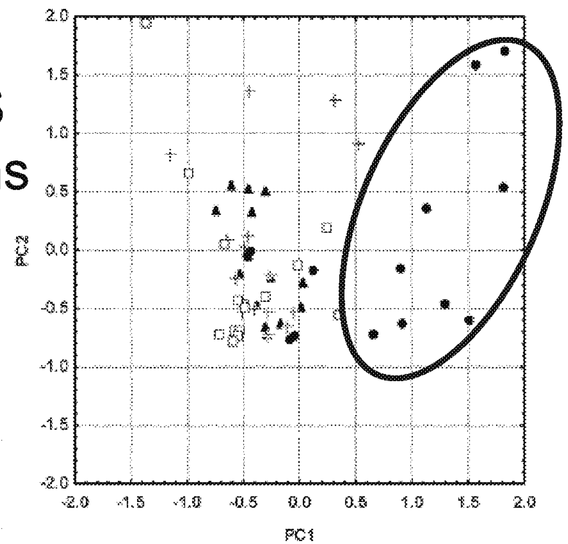
Conder *et al.* ,2008. *ES&T.* 42:995-1003



Blaine *et al.*, 2014a. *ES&T.* 48:7858–7865.

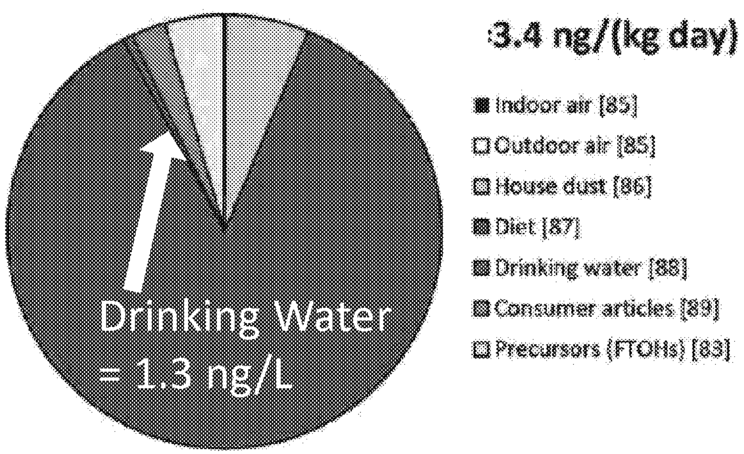
PFAS Exposure through Food

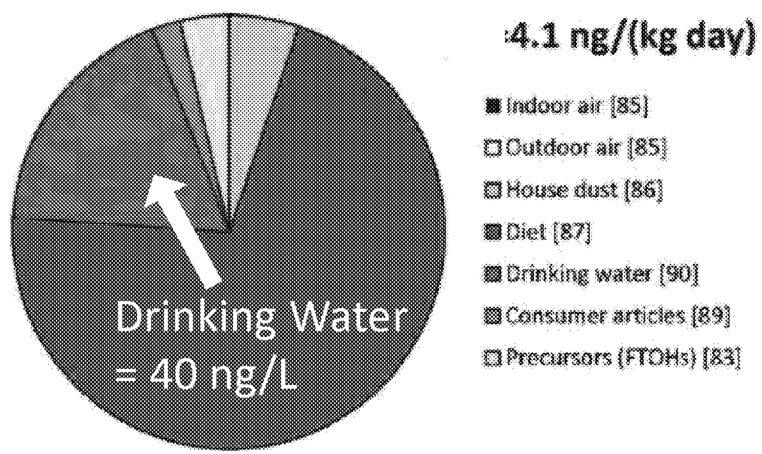
In Poland, higher PFAS body burdens linked to dietary fish intake‡

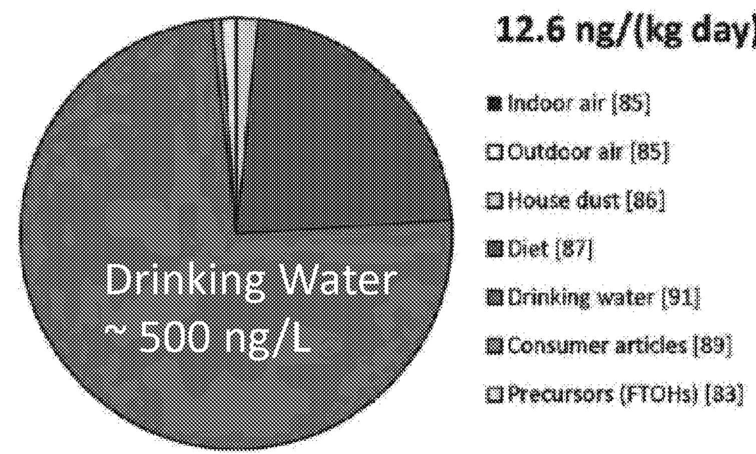


When drinking water is impacted, that is the dominant source of exposure. When it is *not* impacted, diet is likely dominant (at least for PFOA).*

But…are contributions from diet really the same as other communities when the *local environment* is contaminated?







‡ Falandysz *et al.*, *ES&T*. **2006**. 40:748-751

* Vestergren and Cousins, *ES&T*. **2009**. 43:5565-5575

Aim 2 Tasks

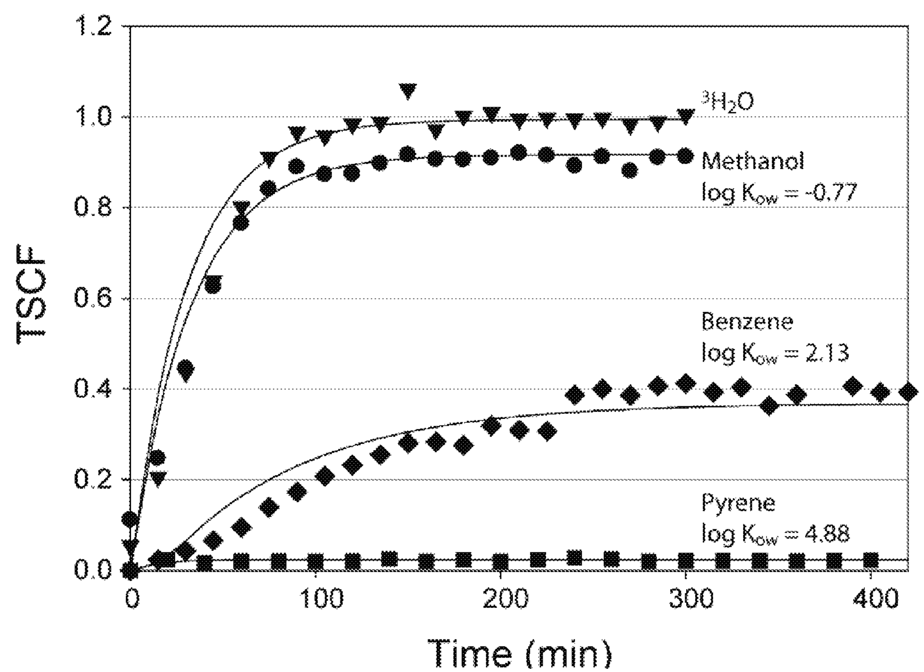
- *Task 2a:* Evaluate uptake of overlooked PFASs into plants via pressure chamber and greenhouse experiments

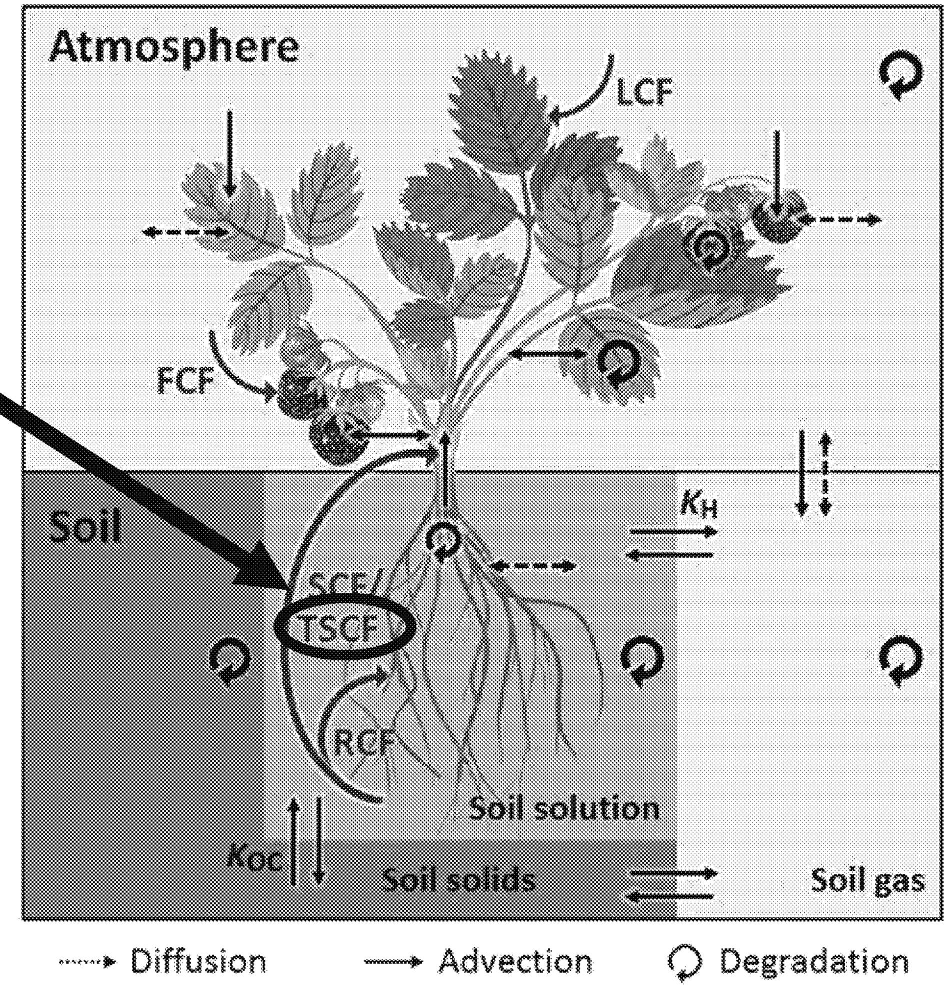
- *Task 2b :* Measure PFAS levels in locally grown/caught/raised food in PFAS-impacted areas and compare to the same foods obtained from non-impacted areas.

Task 2a: Plant Uptake Studies

Transpiration Stream Concentration Factor (TSCF) =

$$\frac{\text{Conc. in xylem sap(mg/L)}}{\text{Conc. in exposure solution(mg/L)}}$$



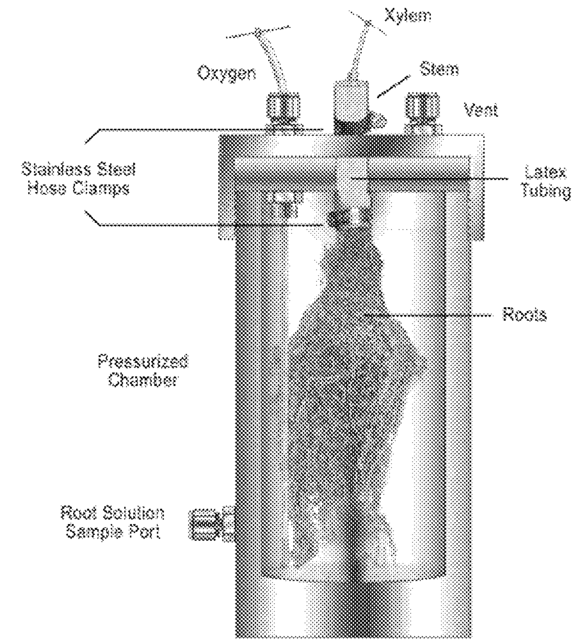


Dettenmaier *et al.*, 2009 *ES&T*; Doucette *et al.* 2018, *ET&C*.

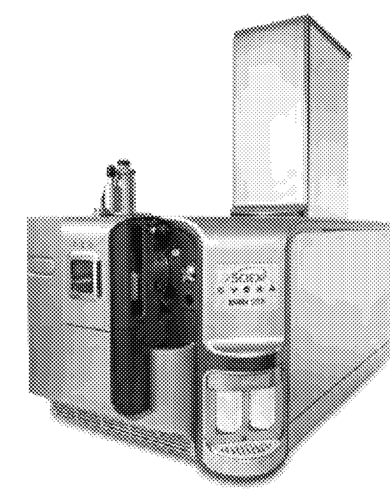
Task 2a: Pressure Chamber Experiments

Approach:

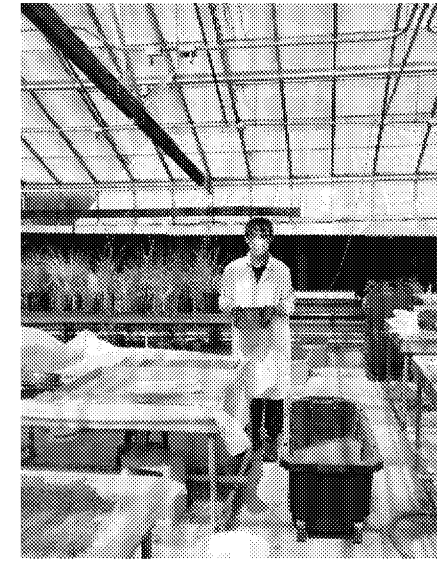
- Detopped plant sealed in pressure chamber
- Root solution spiked with PFASs
- Transpiration simulated by O_2 pressurized chamber
- Xylem sap collected (over time) and analyzed for PFASs
- Root solution collected (over time) and analyzed for PFASs
- Calculate TSCF



We predict that smaller (shorter chain, earlier C18 LC retention) PFASs will have *higher TSCFs* and therefore would be *more likely* to accumulate in above-ground plant mass.



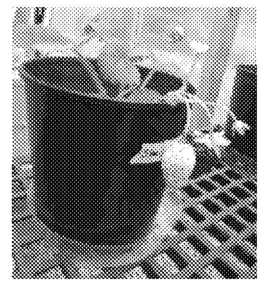
Task 2a: Greenhouse Experiments

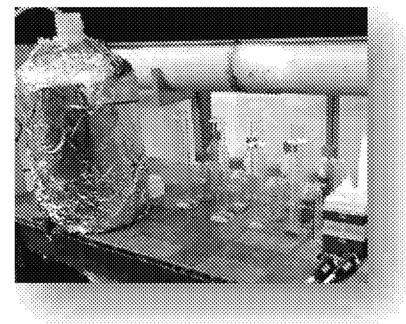


All plants hand irrigated with PFAS-impacted water (supplement with DI)

<u>Crops</u>

- Leaf (i.e., lettuce)
- Root (i.e., carrot)
- Fruit (i.e., tomatoes)





<u>Dosing Experiments</u>

- Plants grown in top soil collected from NC coastal plain (typically sand or loamy sand texture and <1% organic carbon)
- Four dosing concentrations (high and low concentrations of commercial cocktail; neat and diluted groundwater) plus control, with five experimental replicates per dose
- All plant tissues and samples analyzed by LC-QToF-MS for a broad suite of PFASs while enabling the evaluation for potential metabolic transformation of any polyfluorinated compounds

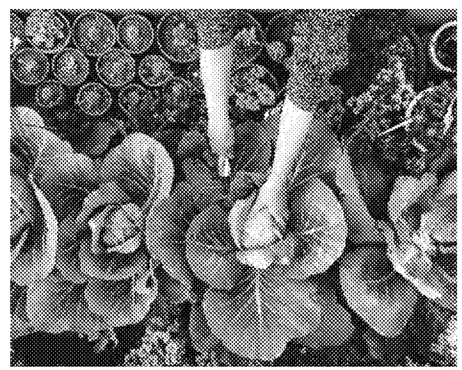
Task 2b: PFASs in Local foods

- Two communities: Michigan (Parchment) and North Carolina (Fayetteville)
    - Also “control” communities where PFASs are known to be low/non-detect in soil/water
- Field-collected plant samples and animal-based foods will be collected from each community and analyzed for PFAS content
    - Working with state and community leaders to invite residents to provide samples of locally grown/raised/caught food they normally consume (fruits, vegetables, eggs, milk, etc.)
    - May include home-grown foods (in conjunction with Aim 3) but also local agricultural products (i.e., Farmers’ Markets)

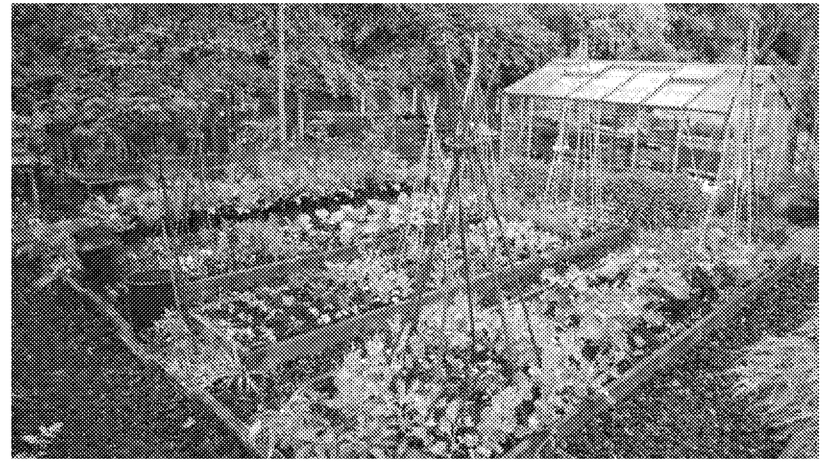
Goal: assess importance of food from PFAS-impacted areas for PFAS exposure and what types of foods are more important for which PFASs.

Task 3. Exposure Assessment

Overall Goal: Assess the relative role of drinking water, diet, and the indoor environment in determining exposure for communities impacted by differing sources of PFASs.

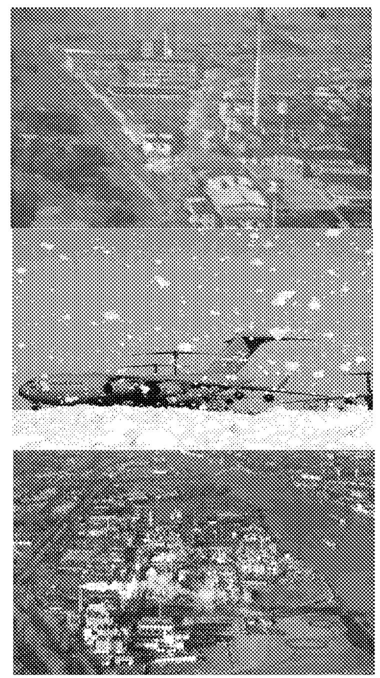


Do Not Eat Advisories



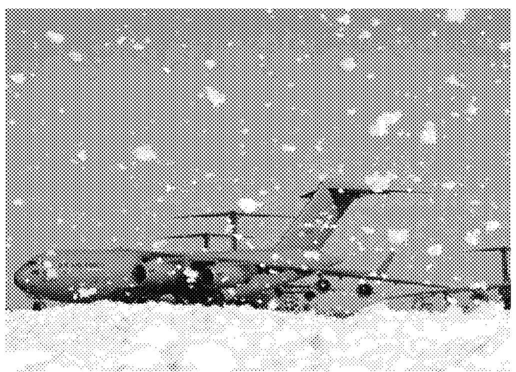
Hypothesis 3a

Differences in PFAS serum levels reflect the differences in drinking water exposure, whereas similarities are consistent with residential and/or consumer product-derived exposure.

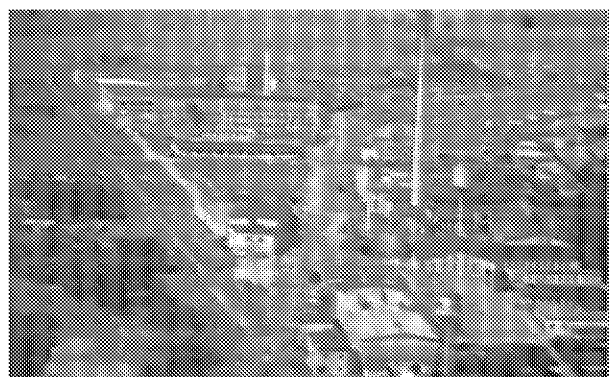


Task 3a: Fingerprinting

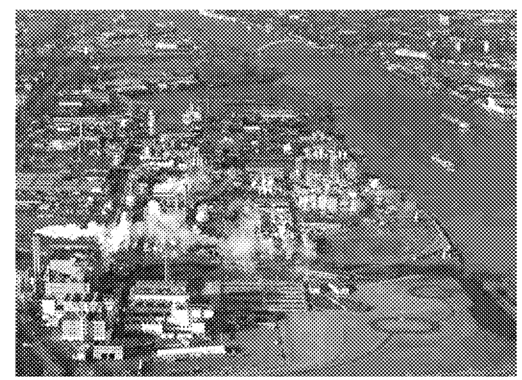
Characterize fingerprints of PFAS mixtures in drinking water and serum collected from three PFAS-affected populations with differing sources of contamination in the U.S.



AFFF (CO)



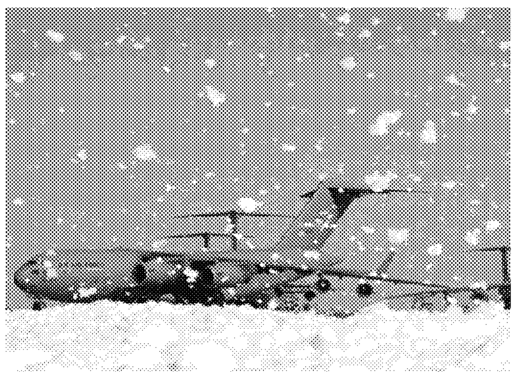
Paper Mill (MI)



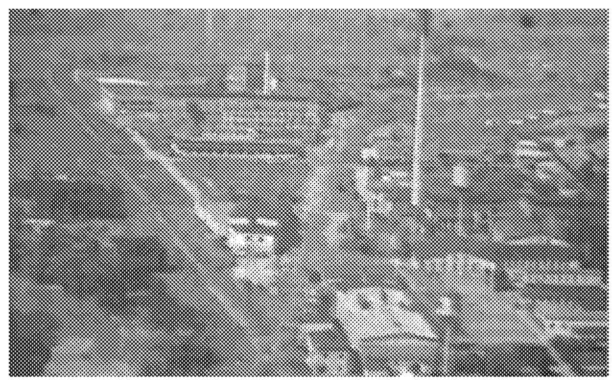
Chemical Manufacturing (NC)

Task 3a: Fingerprinting

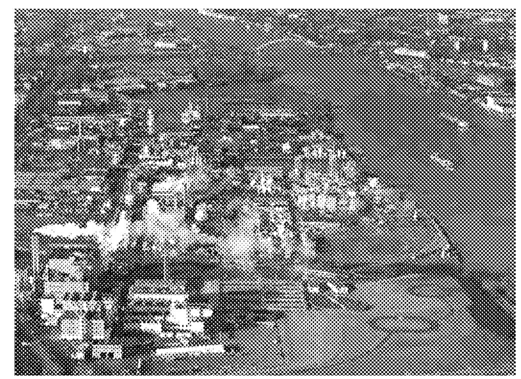
- **Drinking water:** Public water system and private well data from the three communities → PCA
- **Serum:** Individual-level data from biomonitoring studies in the three communities → PCA in parallel analyses



AFFF (CO)



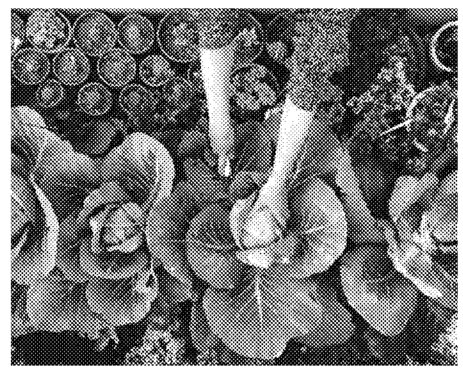
Paper Mill (MI)



Chemical Manufacturing (NC)

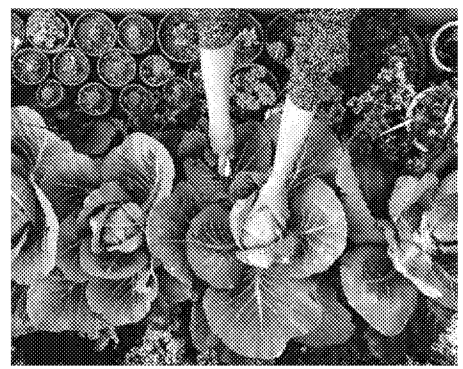
Hypothesis 3b

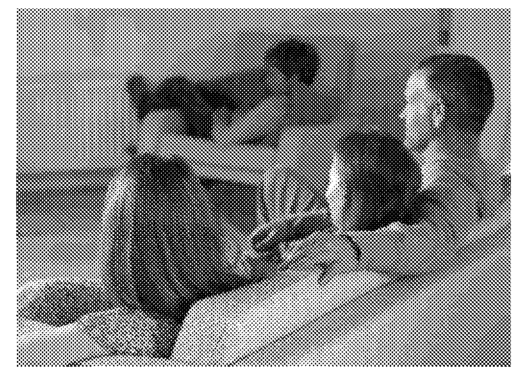
After drinking water, the primary contributors to PFAS exposure will be local food, followed by residential and/or consumer product-derived exposure.



Task 3b: Source Contribution

Quantify relative contributions of drinking water, consumption of local foods, and the indoor environment to PFAS exposure among a PFAS-affected community in Michigan.

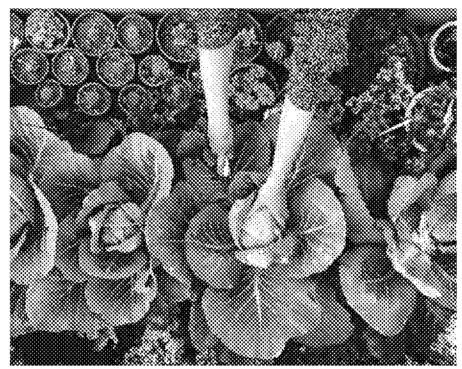




Task 3b: Source Contribution

Predictive (Backward) Modeling

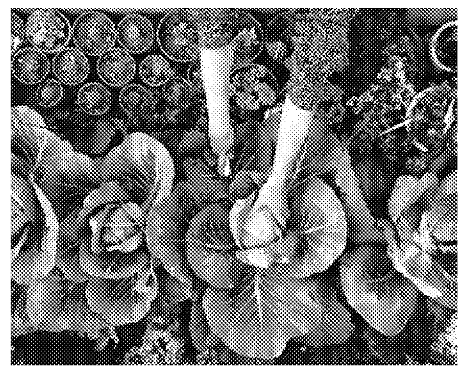
- Associations of serum with drinking water and diet
- Associations of serum with wristbands, air, and dust



Task 3b: Source Contribution

Exposure (Forward) Modeling

- Drinking water
- Diet
- Indoor Environment



Advisory Board

Name	Organization	Current Position	Sector
Ginny Yingling	MN Dept. of Health	Hydrogeologist	State Govt.
Kory Groetsch	MI Dept. of Health and Human Serv.	Env. Pub. Health Div. Dir.	State Govt.
Sandy Mort	NC Dept. of Env.Quality	Env.Toxicologist	State Govt.
Kristy Richardson	CO Dept. of Pub. Health and Env.	State Epidemiologist	State Govt.
Andrea Amico	Testing for Pease	Founder	Community Org.
Cody Angell	Michigan Demands Action	Founder	Community Org.
Kemp Burdette	Cape Fear River Watch	Riverkeeper	Community Org.
Susan Gordon	Fountain Valley Clean Water Coalition	Farmer	Community Org.
Robin Vestergren	IVL	Exposure Scientist	Consultant/Scientific Community
Jennifer Field	Oregon State University	Env. Chemist	Scientific Community
James Hatton	Jacobs	Env. Engineer	Consultant/Scientific Community
Elsie Sunderland	Harvard University	Exposure Scientist	Scientific Community

- Advisory Board (AB) to meet in-person once/year (plus one virtual meeting per year) to provide input and direction on research, but also communications with stakeholders
- Community members will be paid for their time
- In-person meetings will rotate through the three impacted communities (NC, MI, CO)

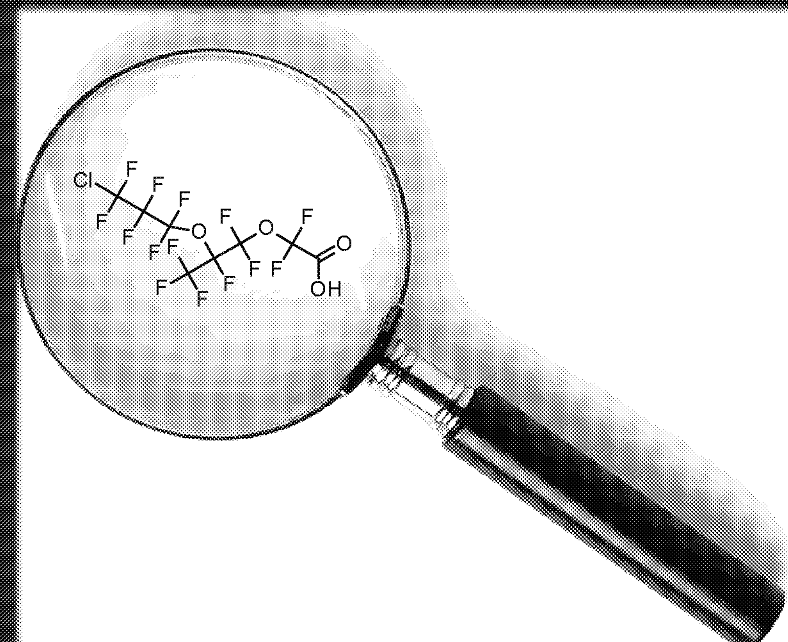
Project Schedule

Project Task	Year 1	Year 2	Year 3
AIM 1			
Task 1a. Sorption equilibrium parameters			
Task 1b. Kinetic sorption parameters			
Task 1c. Vadose zone transport			
AIM 2			
Task 2a. Screening for plant uptake			
Task 2b. Field food uptake assessment			
AIM 3			
Task 3a. Serum and Water Source Signature Characterization and Comparison			
Task 3b. Quantify relative source contributions			
Project Overview (°), Execution Plan (+), and End of Project Debrief (¤)	° +		¤
Annual Progress Reviews (†), and Final Technical Reports (*)		†	† *

Questions?

High Resolution Mass Spectrometry (HRMS) investigations of PFAS and Opportunities for Collaboration

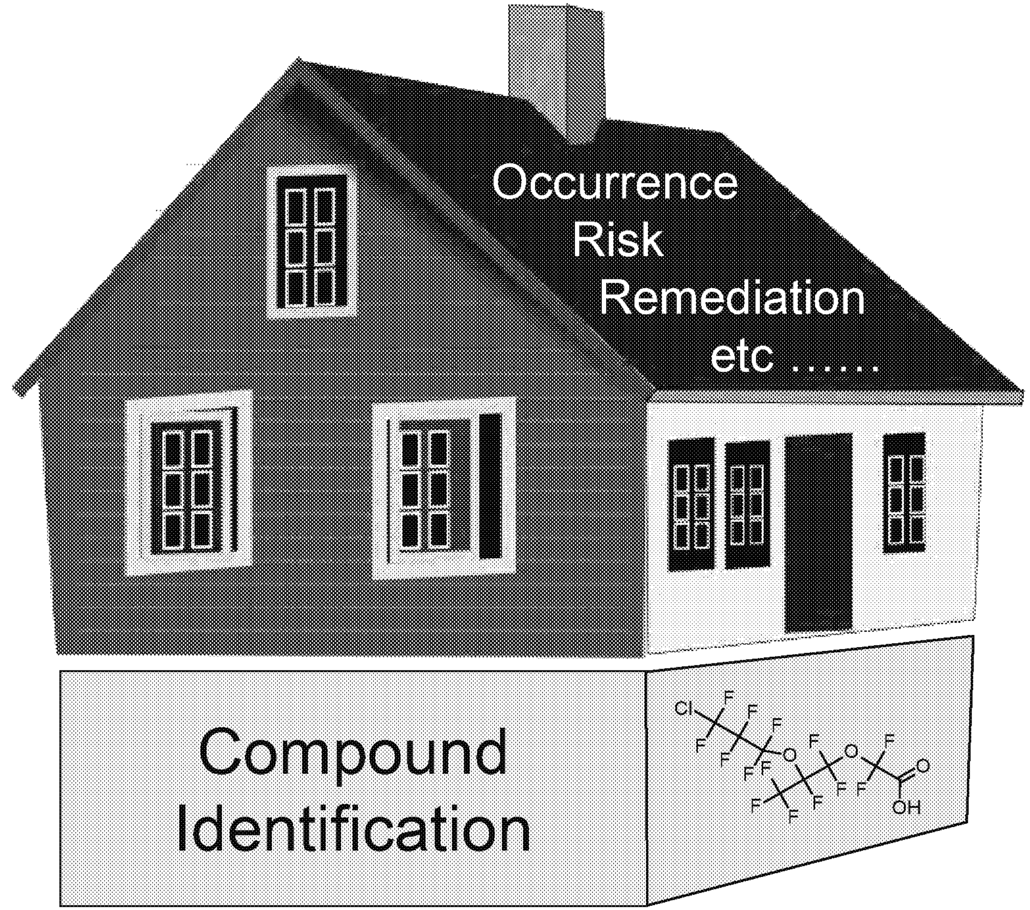
Mark Strynar
US EPA
Office of Research and Development (ORD)
National Exposure Research Laboratory (NERL)



September 5th, 2019
National Priorities: Per- and Polyfluoroalkyl Substances Grant Kickoff Meeting

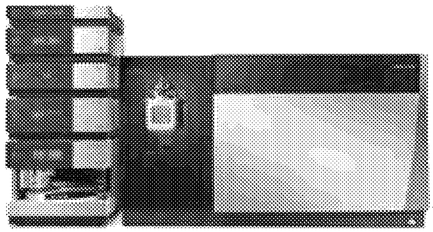
PFAS identification basis

Foundation: First step
Critical for home
Necessary
Everything else is built upon it



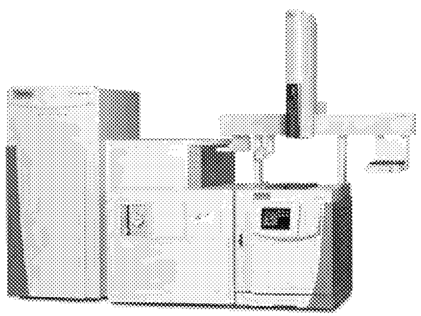
HRMS equipment RTP

IN HOUSE

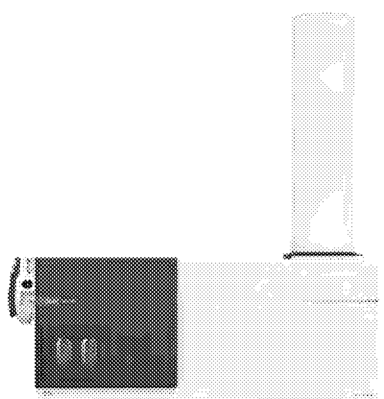


Thermo
LC-Orbitrap Fusion
Strynar/McCord

Pending Equan system add-on

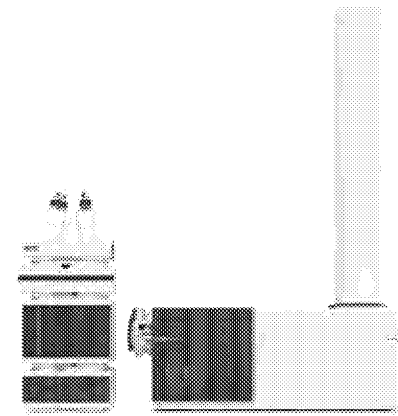


Installed 8-19
Thermo
GC-Orbitrap Q Exactive
Newton

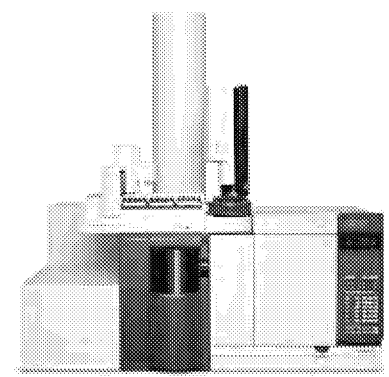


Agilent
6530 LC-QTOF
Ulrich

IN PROCESS



Pending purchase; replacement of old Agilent TOF
Agilent
6546 LC-QTOF
McCord



Pending CRADA delivery
Agilent
GC-QTOF
Clifton

In house PFAS methods RTP

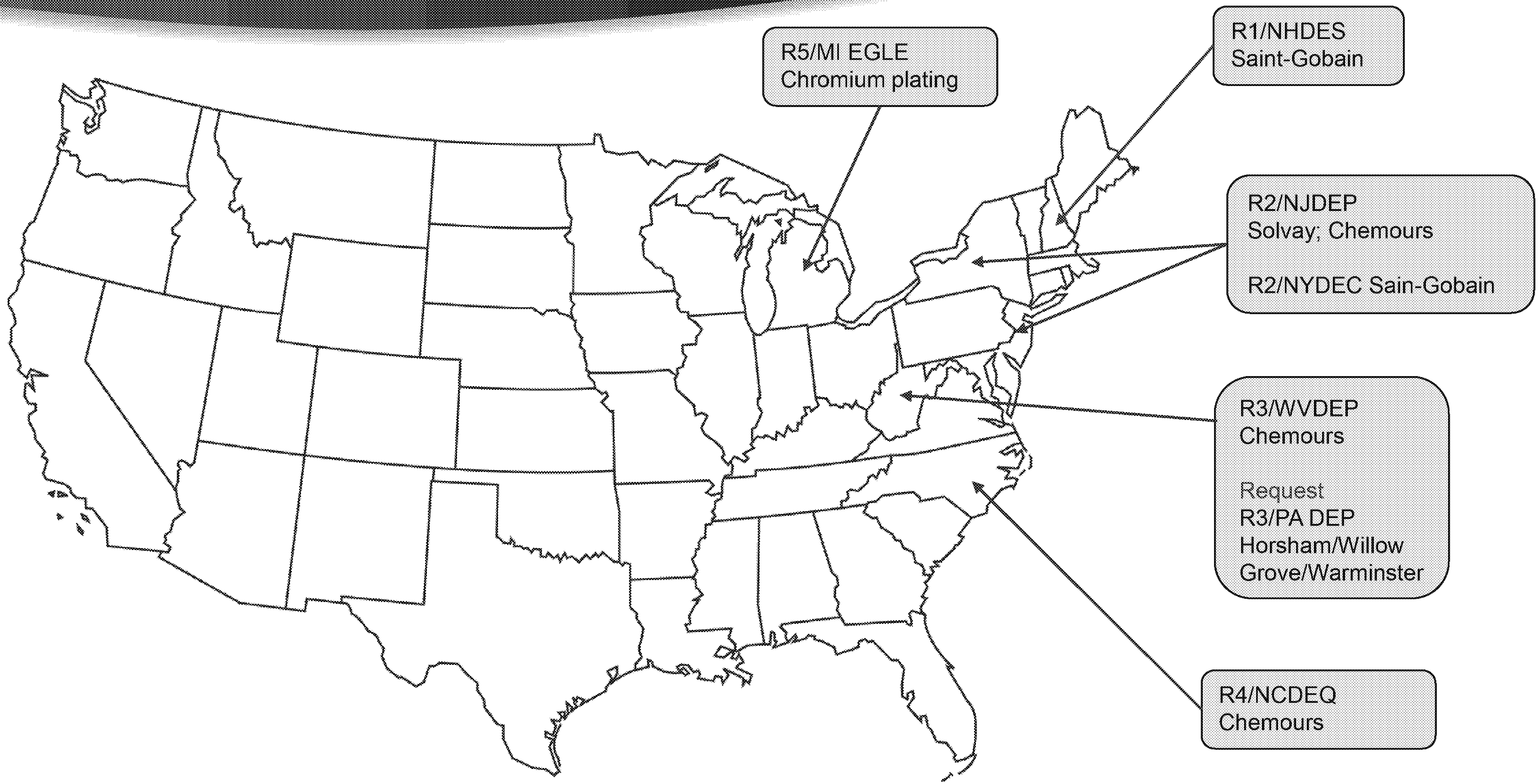
SOPs or published

- Water: waste, surface, ground, drinking
- Solids: soil, sediment, house-dust
- Fish Tissue: muscle, liver, whole fish homogenate, serum, eggs, zebrafish larvae
- Dosed Rodent Tissues: serum, liver, kidneys, brain, whole-pup
- Human Biological: serum

In Process

- Human Biological: urine
- Industrial Products: dispersions, AFFF
- Impinger Sampler: XAD resin, aqu. Impingers (boric acid, sodium hydroxide, DI)

Active Region/State Collaborations



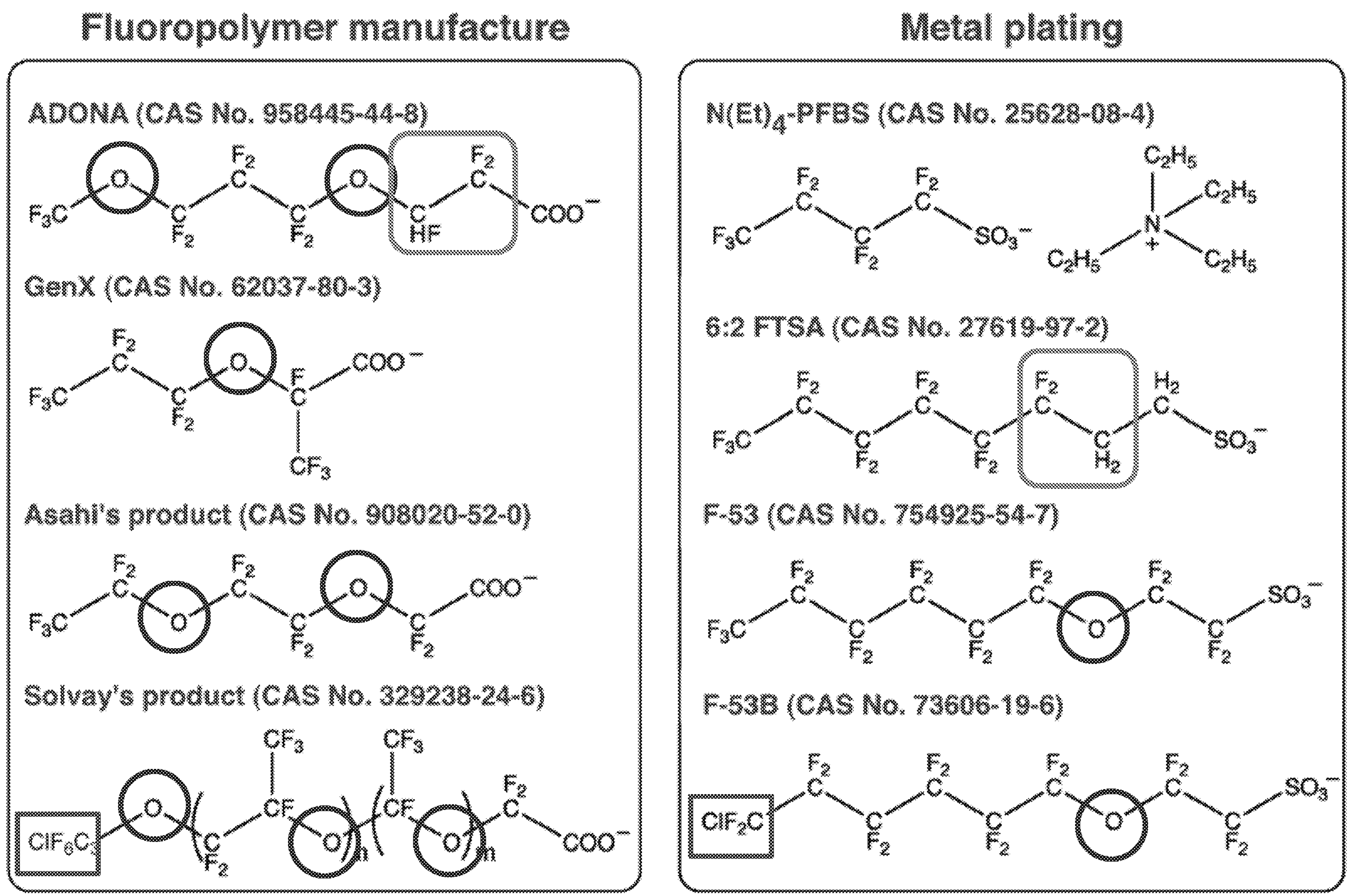
Post PFOA Stewardship Agreement

Ethers/Polyethers

Polyfluorinated

Chlorinated

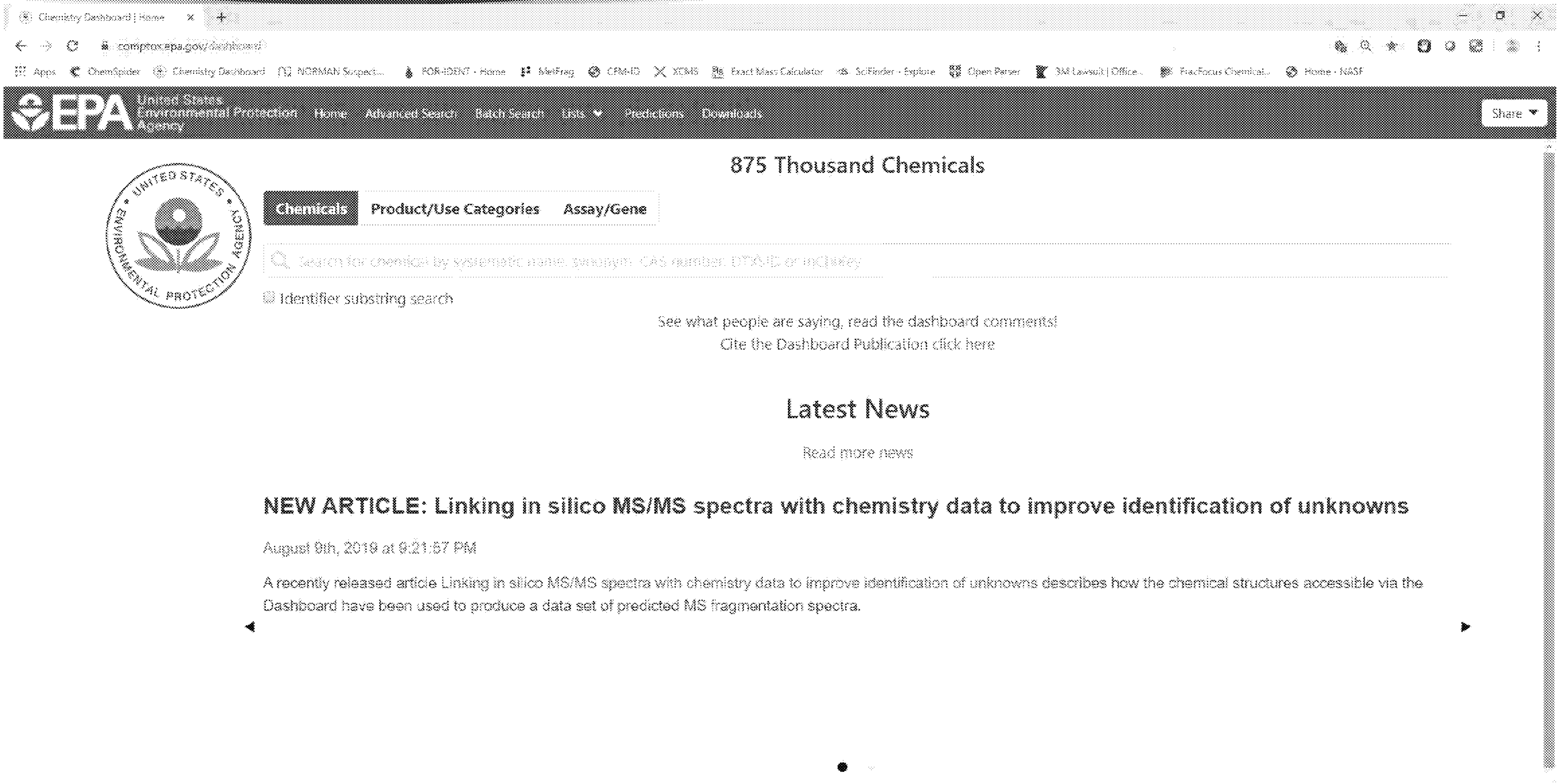
Manufacture
Use
Release



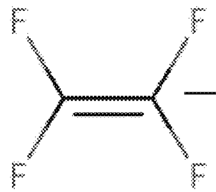
Wang et al. *Environment International*, 2013, *60,* pp 242–248

EPA's Chemistry Dashboard

https://comptox.epa.gov/dashboard



Polymer Processing Aid (PPA)

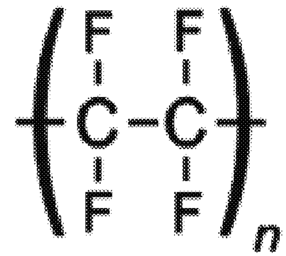


TFE

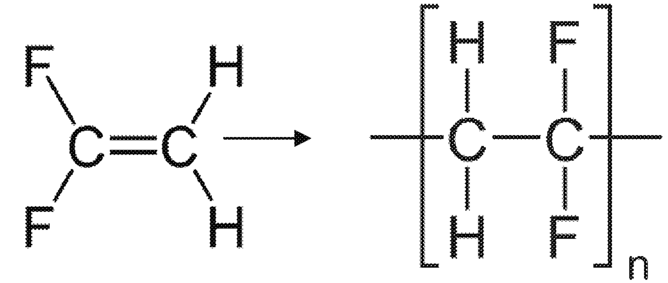
PPA:
PFOA; APFO

GenX
ADONA…

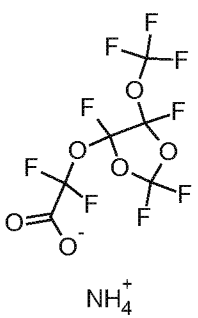
PTFE (Teflon)



PVDF



Solvay/Miteni

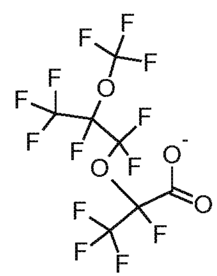


Molecular Formula (Anion): $C_6F_9O_6^-$
Monoisotopic Mass: 338.955665 Da

https://echa.europa.eu/registration-dossier/-/registered-dossier/5331/6/2/2

Daikin 510774-77-3

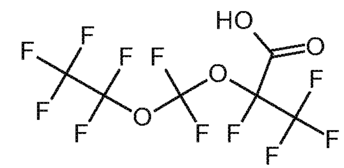
CAS 510774-77-3



https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID70896654

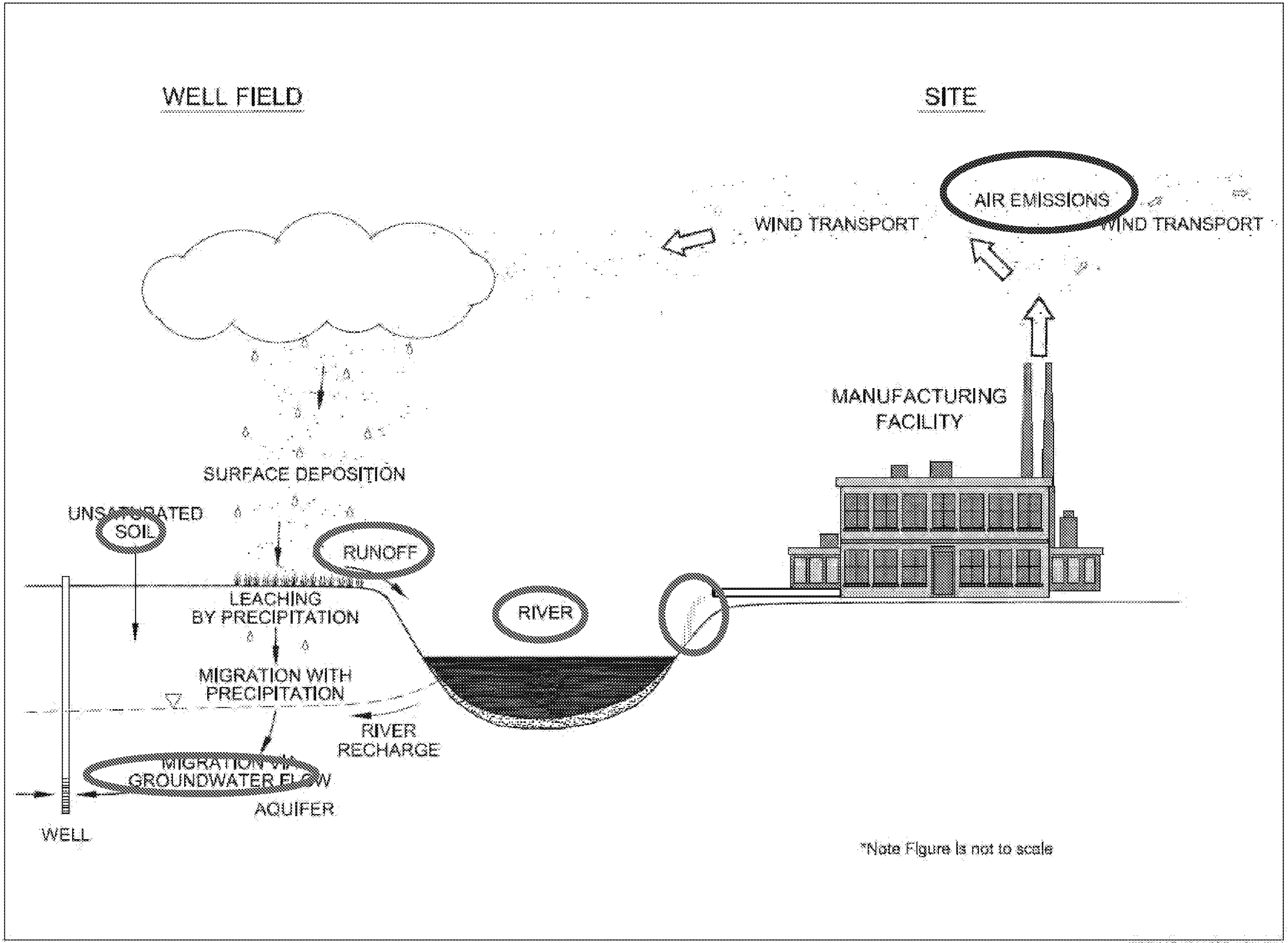
Molecular Formula: $C_7F_{13}O_4^-$
Monoisotopic Mass: 394.9594 Da

KCKK Polymer Processing Aid



Molecular Formula: $C_6HF_{11}O_4$
Monoisotopic Mass: 345.969919 Da
[M-H]-: 344.962642 Da

Conceptual Model of APFO Emission



Davis et al., 2007 Chemosphere (67) 2011-2019
"Transport of ammonium perfluorooctanoate in environmental media near a fluoropolymer manufacturing facility"

NTA Samples to compare:

Upstream vs. downstream
Pretreatment vs posttreatment
Close vs distant
Upwind vs downwind
Surface vs deep
Etc….

MM5 Impinger Schematic/Industrial Process

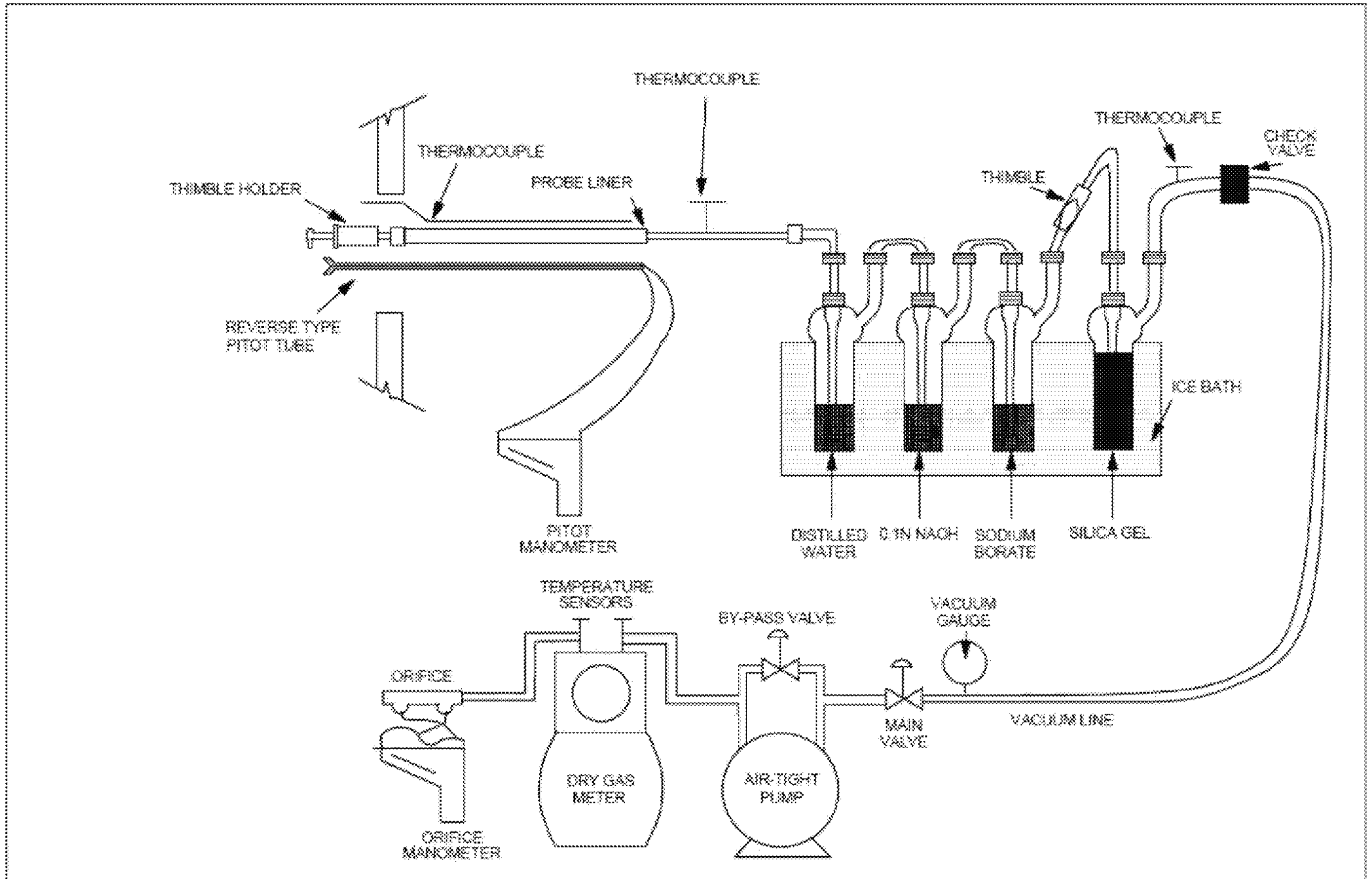
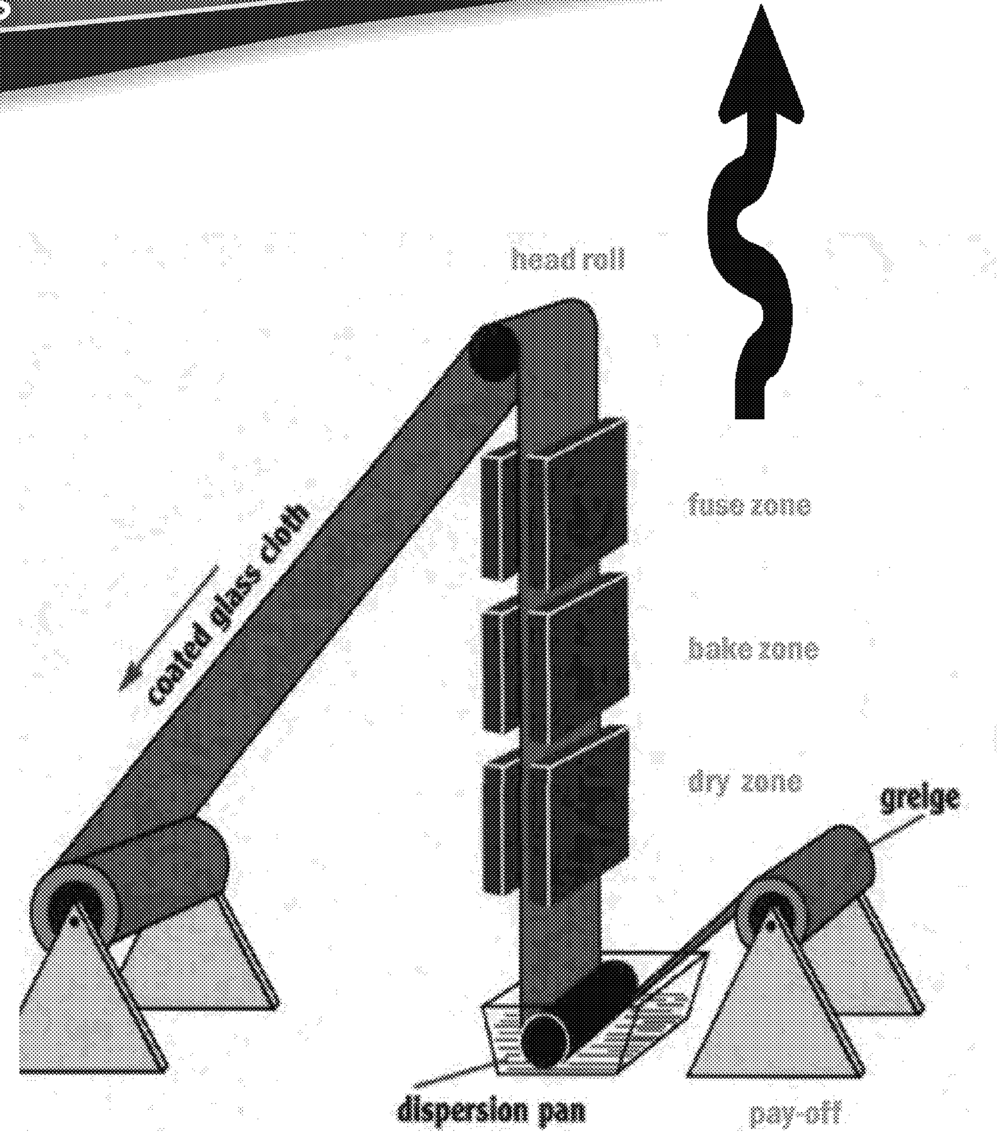


FIGURE 3-1
MODIFIED EPA METHOD 5
PFOA SAMPLING TRAIN



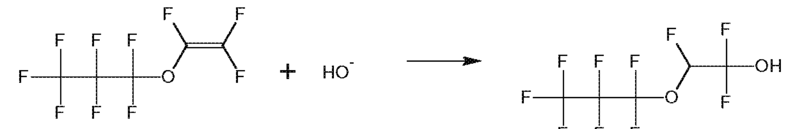
PTFE dispersion textile (kevlar/fiberglass) coating

KNOWN PFAS CONVERSION REACTIONS

VINYL ETHERs IN BASE TO 1H SUBSTITUTED

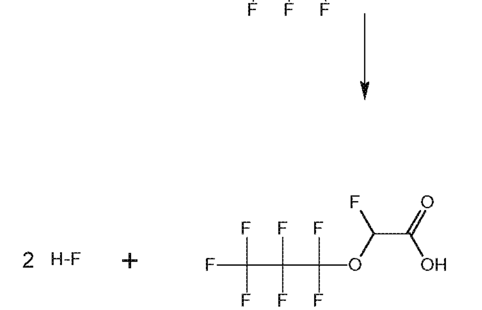
KNOWN

PPVE



CAS 1623-05-8
DTXSID0061826

PPVE

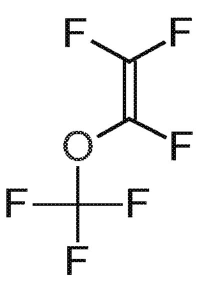


Molecular Formula: $C_5H_2F_8O_3$
Monoisotopic Mass: 261.98762 Da
[M-H]-: 260.980343 Da

Basic impinger traps, vinyl ethers would be converted to corresponding acid

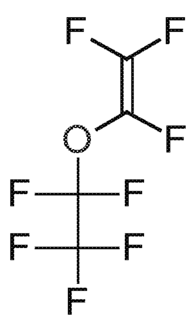
OTHERS POSSIBLE

PMVE

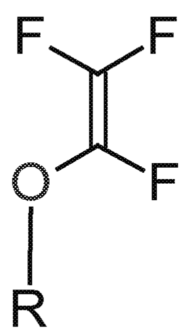


CAS: 1187-93-5
DTXSID3051599

PEVE



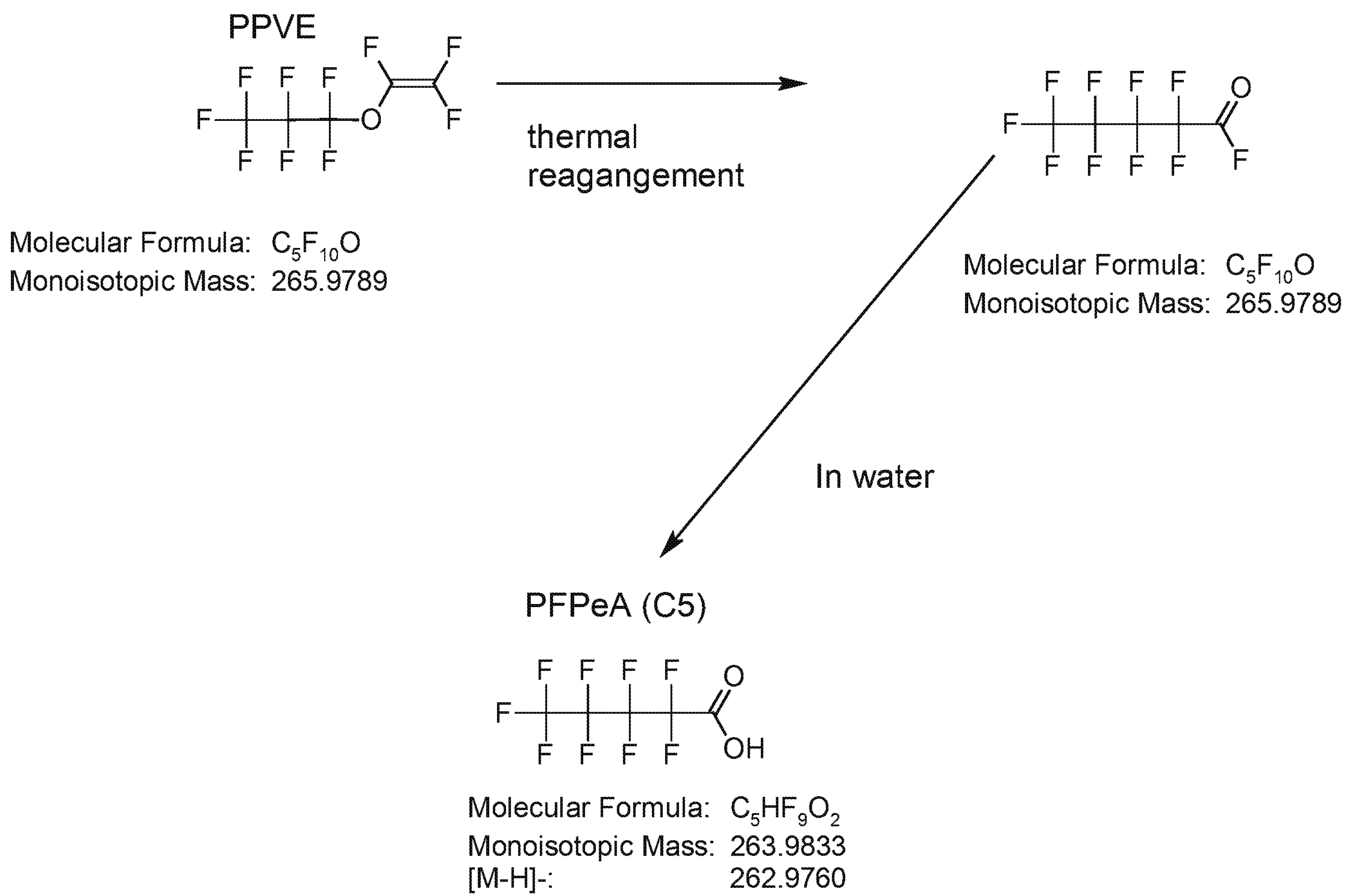
CAS: 10493-43-3
DTXSID1075305



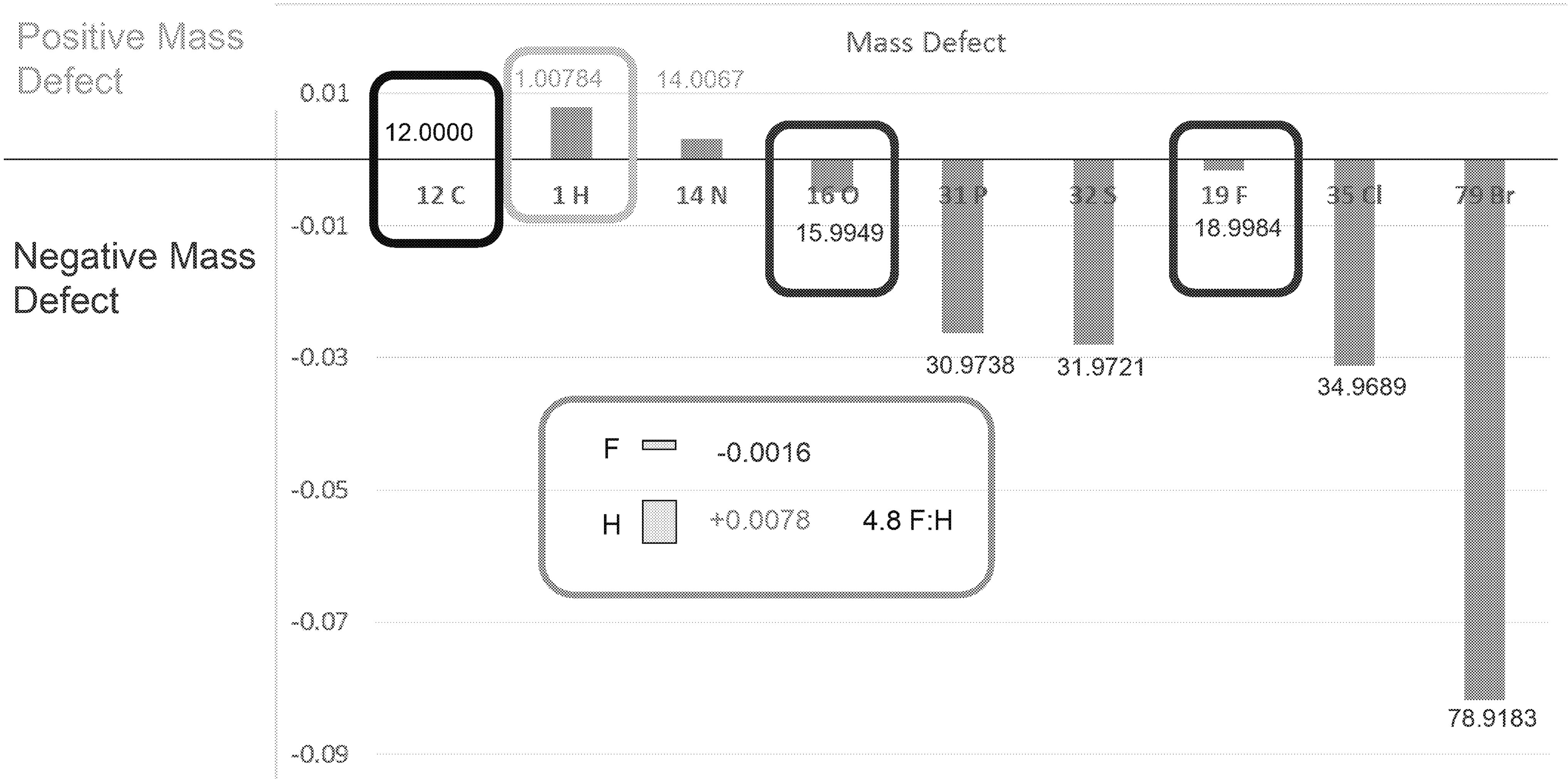
R = any moiety; Nafion BP1

THERMAL REARANGEMENT TO ACYL-FLUORIDE

Source of PFPeA (C5) in Fayetteville, NC



Isotope Signatures: Negative Mass Defect



Not as easy for AFFF- slightly positive mass defect

Environmental Science & Technology

November 2015

Article

pubs.acs.org/est

Identification of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS)

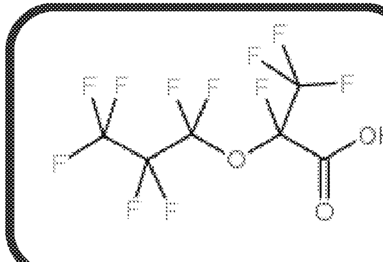
Mark Strynar,*[†] Sonia Dagnino,[†,‡] Rebecca McMahen,[†,‡] Shuang Liang,[†,‡] Andrew Lindstrom,[†] Erik Andersen,[†] Larry McMillan,[§] Michael Thurman,[∥] Imma Ferrer,[∥] and Carol Ball[⊥]

Table 1. Accurate Mass of Polyfluorinated Compounds and In-Source Artifacts Found in Extracted Water Samples

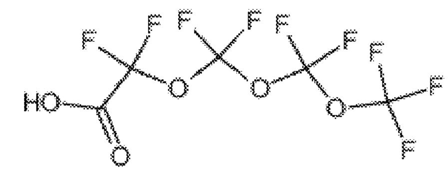
number	formula	CAS no.	name	[M][a]	[M − H]⁻ m/z	[2M − 2H + Na]⁻ m/z	[2M − H]⁻ m/z
Monoether PFECAs							
1	$C_3HF_5O_3$			179.9846	178.9773	380.9438	358.9619
2	$C_4HF_7O_3$			229.9813	228.9740	480.9372	458.9553
3	$C_5HF_9O_3$	863090-89-5		279.9782	278.9709	580.9310	558.9491
4	$C_6HF_{11}O_3$	13252-13-6	undecafluoro-2-methyl-3-oxahexanoic acid	329.9750	328.9677	680.9247	658.9427
5	$C_7HF_{13}O_3$			379.9718	378.9645	780.9182	758.9363
6	$C_8HF_{15}O_3$			429.9686	428.9613	880.9118	858.9299
Polyether PFECAs							
7	$C_7HF_{13}O_7$	39492-91-6	perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	443.9515	442.9442	908.8776	886.8957
8	$C_6HF_{11}O_6$	39492-90-5	perfluoro-3,5,7,9-butaoxadecanoic acid	377.9598	376.9525	776.8942	754.9123
9	$C_5HF_9O_5$	39492-89-2	perfluoro-3,5,7-propaoxaoctanoic acid	311.9681	310.9608	644.9108	622.9289
10	$C_4HF_7O_4$	39492-88-1	perfluoro-3,5-dioxahexanoic acid	245.9764	244.9691	512.9274	490.9455
PFESAs							
11	$C_7HF_{13}O_5S$	66796-30-3[b]		443.9337	442.9264		
12	$C_7H_2F_{14}O_5S$			463.9399	462.9326		

3 Found in human serum Wilmington, NC 2019 (NCSU Kotlarz/Hoppin)

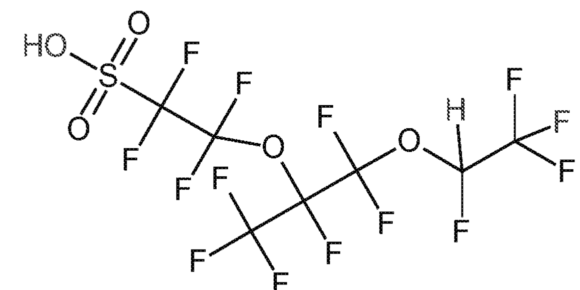
Example Structures



Monoether (6): GenX

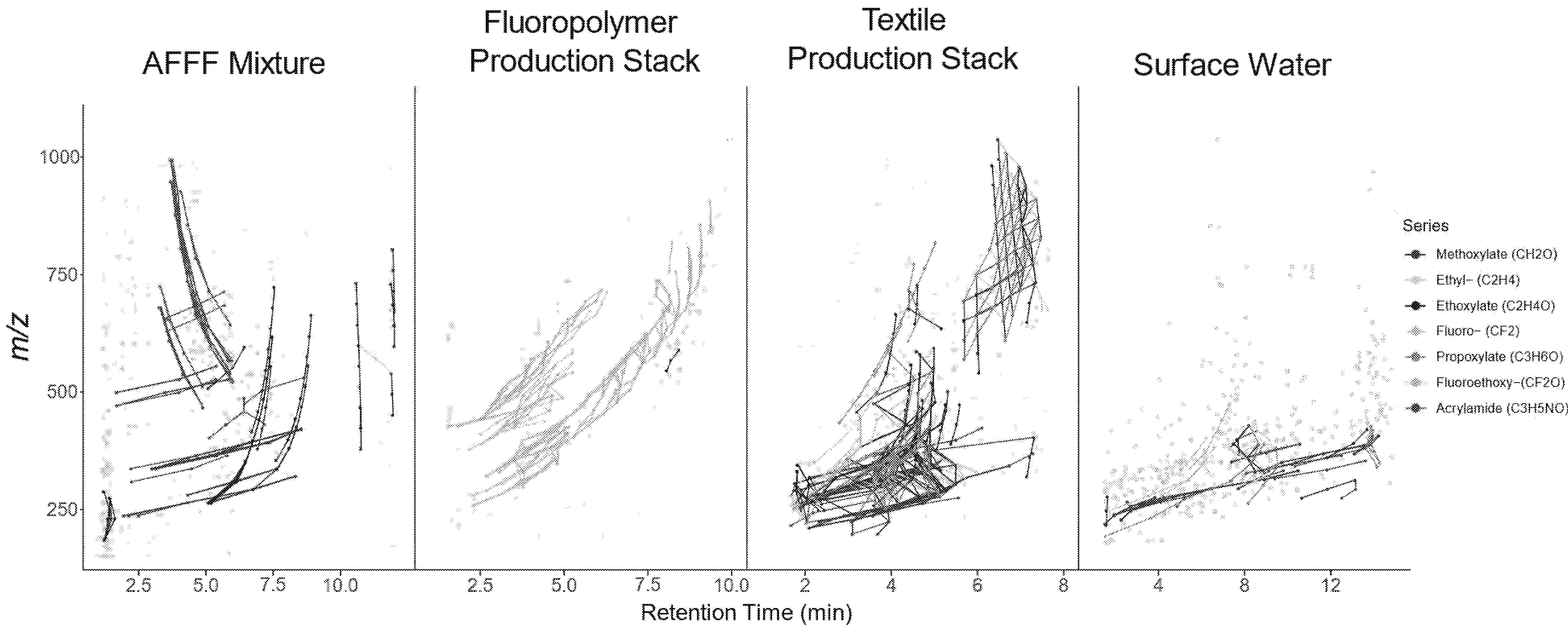


Polyethers (4):

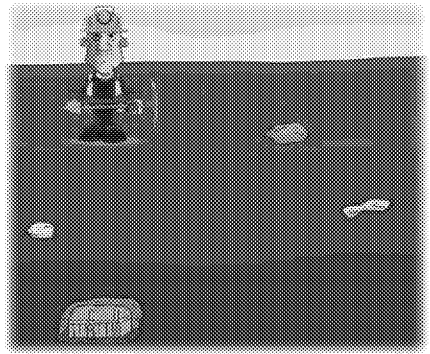


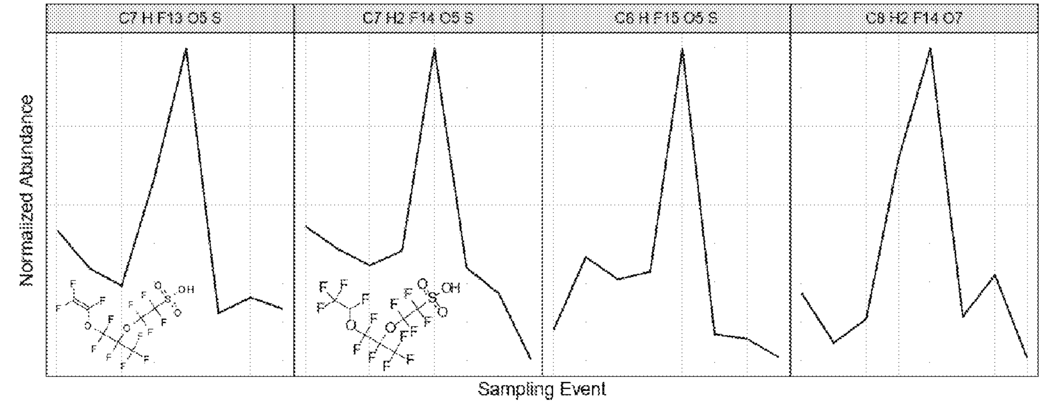
Polyethers sulfonates (2):

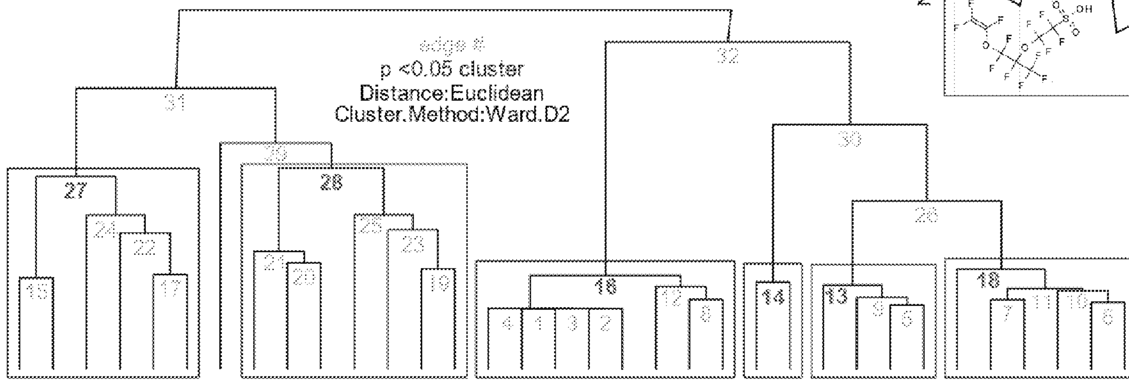
Homologous Series/ Repeating units



Data Mining for What to Focus On





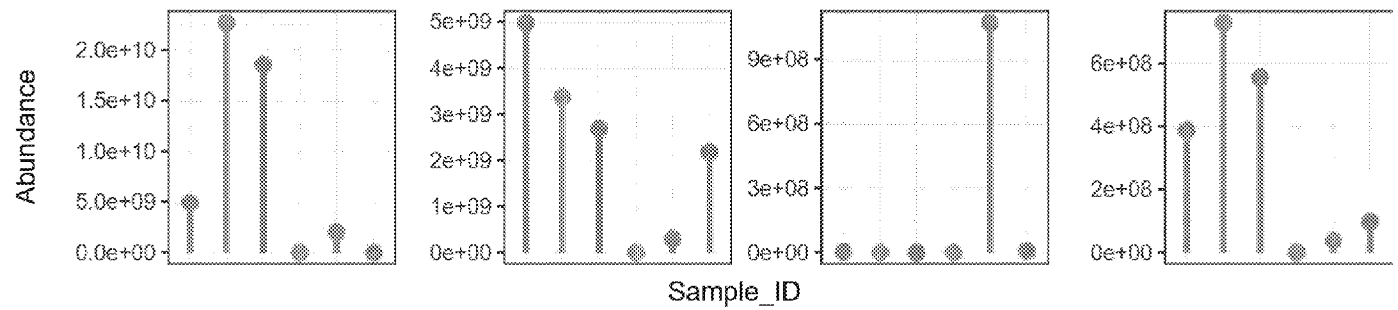


Trends

Clustering

Fingerprinting

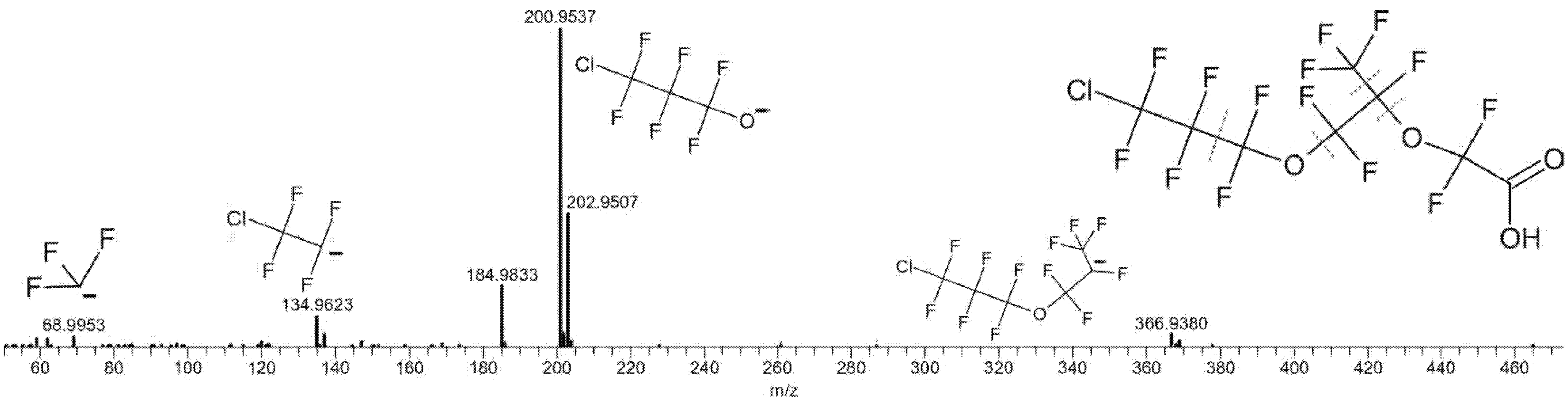
Previously Undiscovered



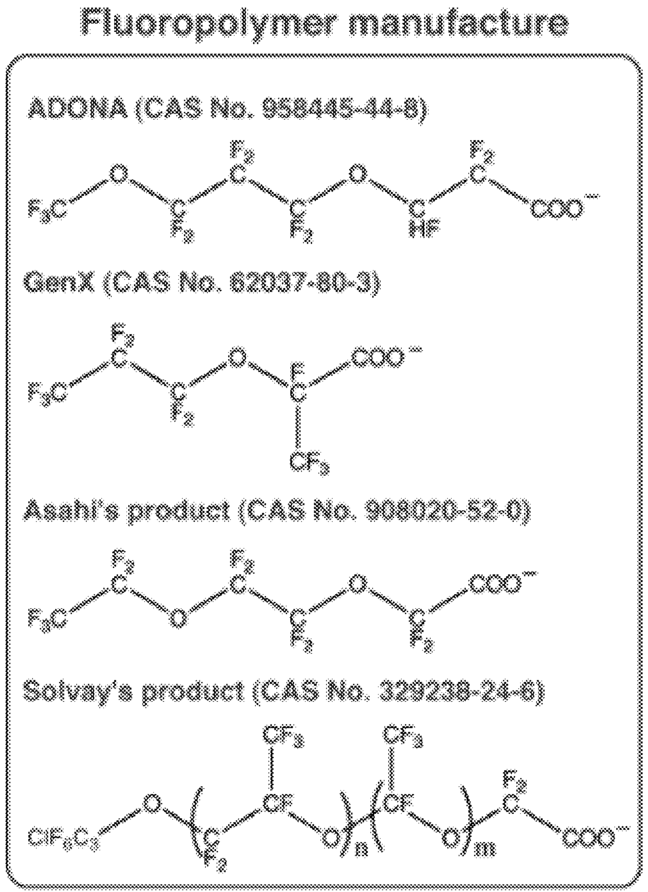
ED_005565_00007863-00079

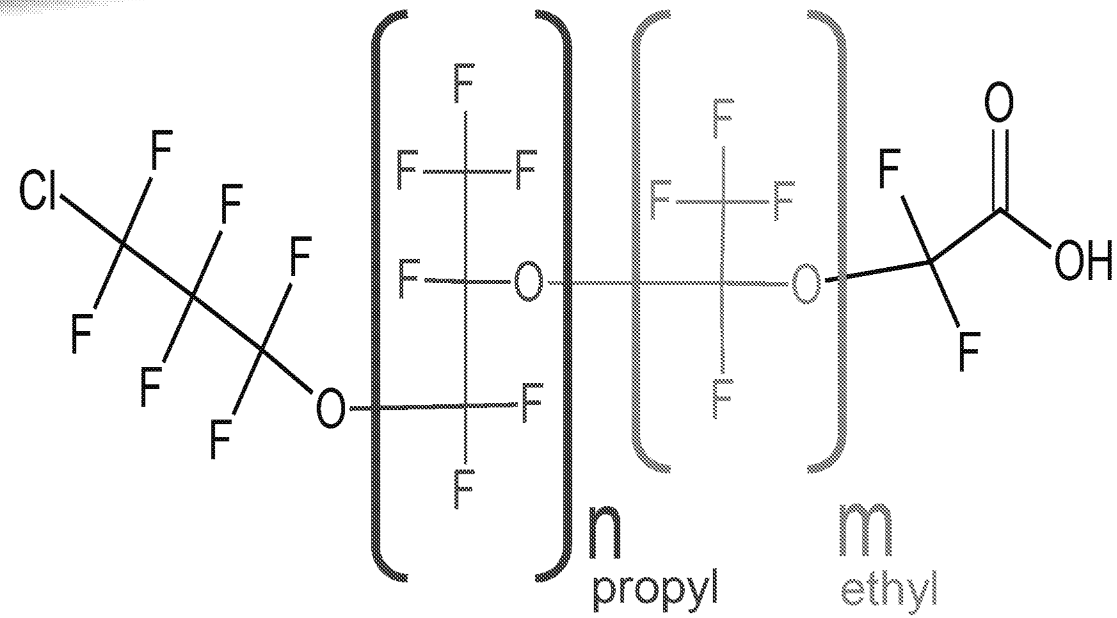
Structural Elucidation by MS/MS

C8 H F14 Cl O4



- Unequivocal assignment of terminal Cl and ether positions based on MS/MS experiments
- Confirmation of Dimers and in-source fragments from prior slide, with additional experiments (not show)





Chloro perfluoro polyether carboxylic acids ClPFPECAs(n,m)

Wang, Z., et al. (2013). Environ. Int. **60: 242.**
EFSA J, 8 (2) (2010), p. 1519, 10.2903/j.efsa.2010.1519

Collaboration Opportunities

Working with Sue Fenton and Mike DeVito (NIEHS/NTP)

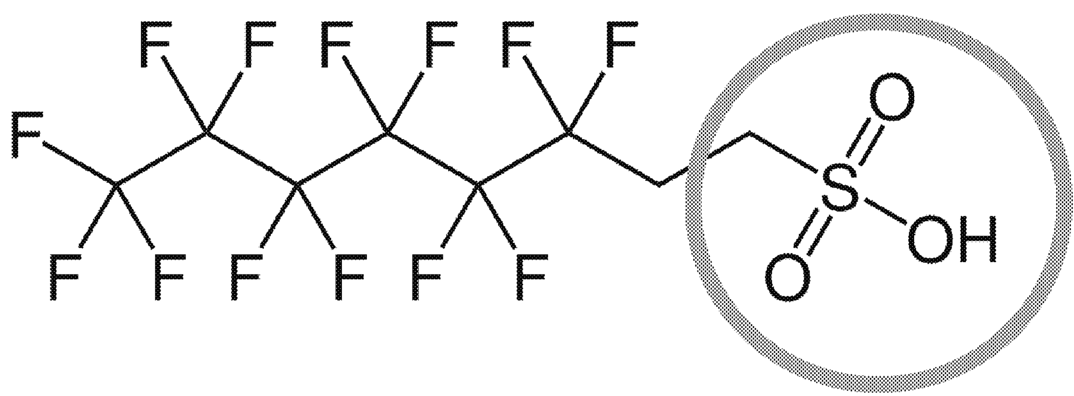
Contemporary AFFF formulations:

- Phos Chek (PFAS free)
- Tridol 3%
- Tridol 6%
- FireAde Milspec 3%
- FireAde Milspec 6%
- Foamtec 3%
- Chemguard 3%
- Chemguard 6%
- Solberg 6%
- Firestopper Concentrate (Wiley)

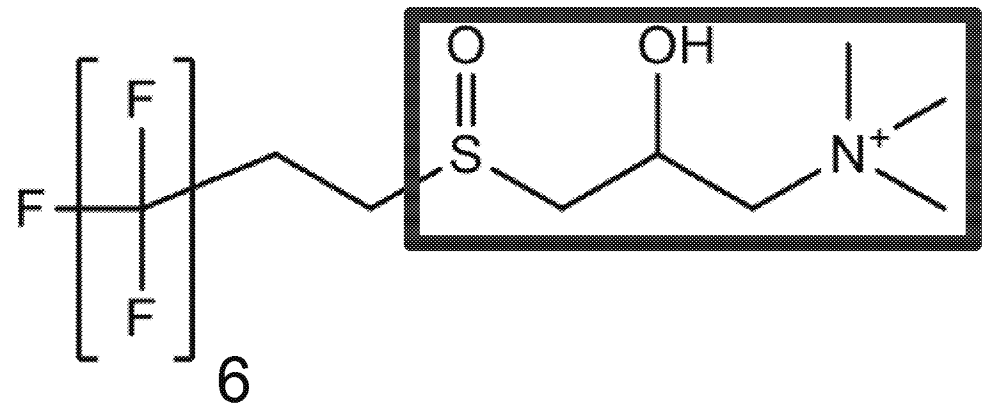
Fluorinated Components

- No fluorinated series detected in any mixture
- Formulations list C6 in product descriptions, all mixtures had detectable 6:2 fluorotelomer surfactants
- Head groups differ

6:2 Fluorotelomer Sulfonate

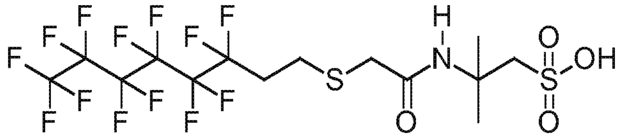


6:2 Fluorotelomer thiohydroxyl Ammonium - sulfoxide

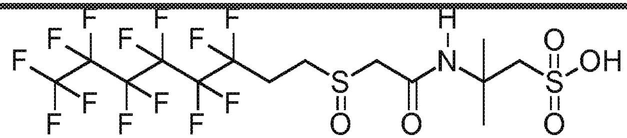


Most Abundant 6:2 Fluorotelomer Species

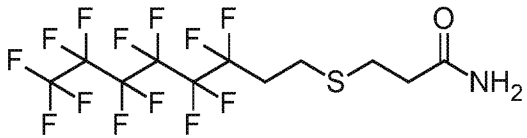
Also found in 1 NH PTFE dispersion



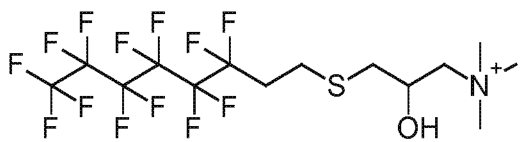
(6:2 FTSAS) 6:2 Fluorotelomer mercaptoalkylamido sulfonate



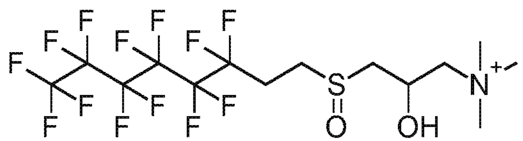
(6:2 FTSAS sulfoxide) 6:2 Fluorotelomer mercaptoalkylamido sulfonate sulfoxide



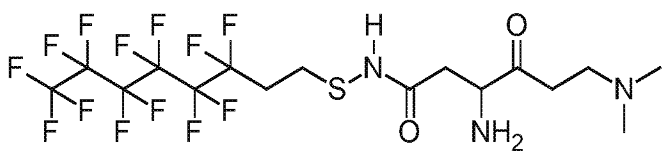
(6:2 FTSPA) 6:2 Fluorotelomer propanamide



(6:2 FTSHA) 6:2 Fluorotelomer thiohydroxyl ammonium



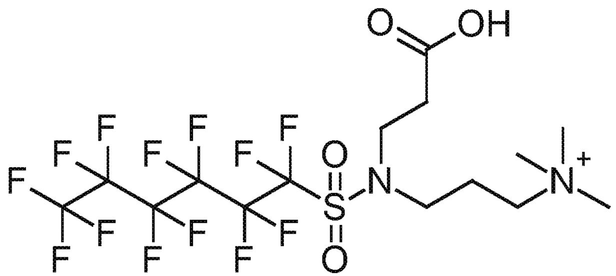
(6:2 FTSHA-O) 6:2 Fluorotelomer thiohydroxyl ammonium sulfoxide



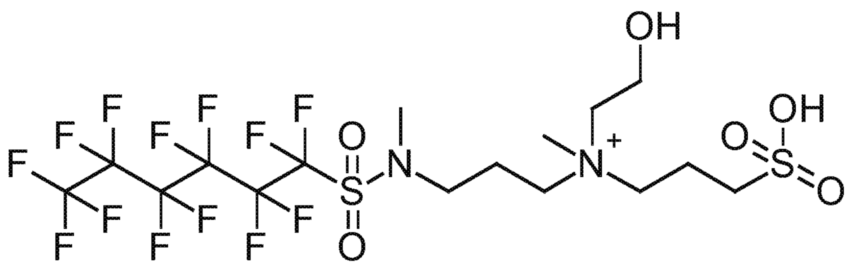
(6:2 FTSAmA) Tentative 6:2 fluorotelomer sulfamido amine

(Unknown 594) Unknown 6:2 Fluorotelomer with mz 594.1101

Additional Species: Perfluoro Sulfonamides

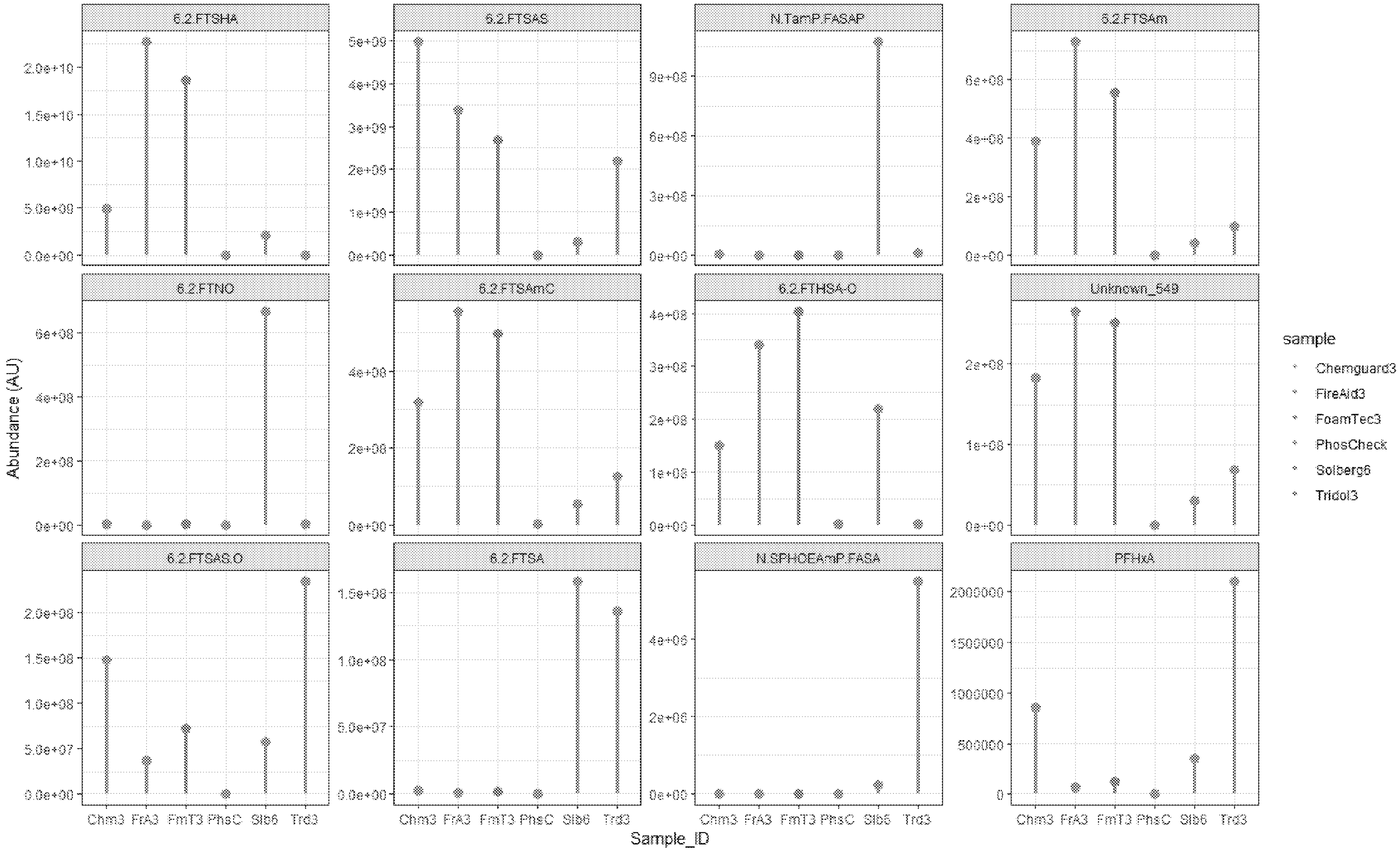


(N-TAmP-FASAP)
N-TrimethylAmmonioPropyl N-Methyl perfluoro Alkane SulfonAmide



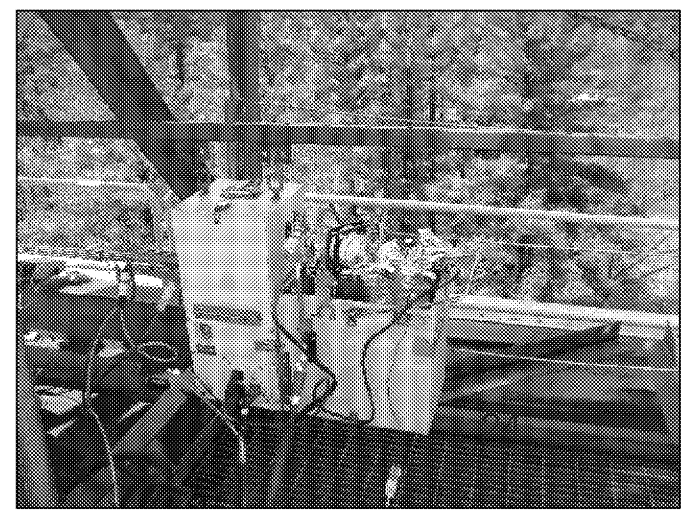
(N-SPOEAmP-FASA)
N-SulfoPropyl(Hydroxyethyl/Methyl) AmmonioPropyl perfluoro Alkane SulfonAmide

C6 Telomers vary between samples



ORD PFAS Incineration Emissions Testing

- AFFF contaminated soil incinerated in Alaska
- Joint study with DoD SERDP Program to characterize emissions
  - Focus on potential Products of Incomplete Combustion (PICs) formation
  - Attempting to characterize volatile/semivolatile and polar/nonpolar PFAS compounds
  - Non Targeted Analyses (NTA) primary focus
- Excellent emissions measurement methods development opportunity
  - Evaluation of multiple methods
  - Targeted PFAS compound emissions measurement data quality
  - Attempt to measure Total Organic Fluorine (TOF) by combustion-ion chromatography (C-IC)
- Collaboration Opportunities?
  - NTA for volatile/semivolatile and polar/nonpolar PFAS compounds (solvent extracts, sorbent traps, etc)
- TOF approaches, including sampling strategies



Slide courtesy of Jeff Ryan NRMRL

Questions?

Contact Information

strynar.mark@epa.gov

919-541-3706

System Toxicological Approaches to Define and Predict the Toxicity of Per and Polyfluoroalkyl Substances

Robert Tanguay

Oregon State University
Department of Environmental and Molecular Toxicology
Sinnhuber Aquatic Research Laboratory
Environmental Health Sciences Center

The Leadership Team

Oregon State University

University of Pittsburgh

ECU

NC STATE UNIVERSITY

Robert Tanguay **Carla Ng** **Jamie DeWitt** **David Reif**

Jennifer Field

Lisa Truong

Overall Objectives

- Study the toxicity of a large collection (430) of volatile and non-volatile PFASs and PFAS mixtures in zebrafish (Tanguay, Truong, Reif)

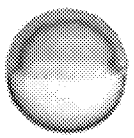
- Conduct developmental immunotoxicity (DIT) studies in mice with the PFAS compounds that are bioactive in zebrafish (Dewitt).

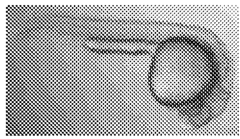
- Create pharmacokinetic models that can explain and predict the concentrations of PFASs in the organs of mice and adult zebrafish as a function of exposure dose and chemical structure (Ng).

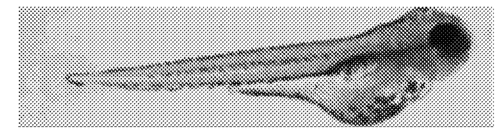
This team responds directly to EPA's solicitation (EPA-G2018-ORD-A1) for information about "PFAS toxicity, modes of action, and physiologically-based pharmacokinetics"

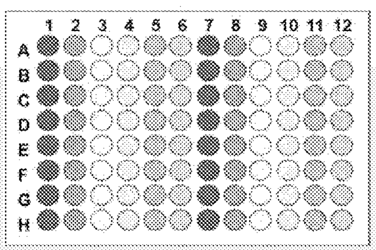
High-Throughput Screening of PFAS compounds

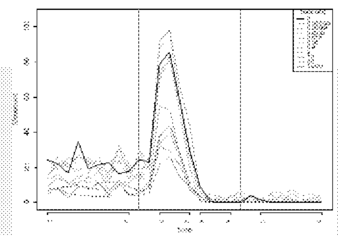
6 hpf 24 hpf 48 hpf 120 hpf





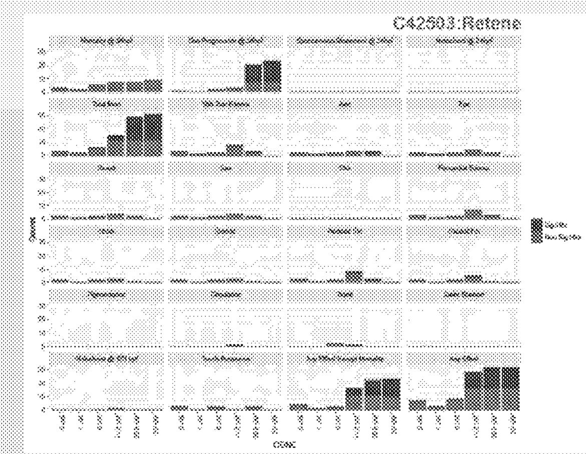






48 hour Tissue Collection
For Body Burden Measures

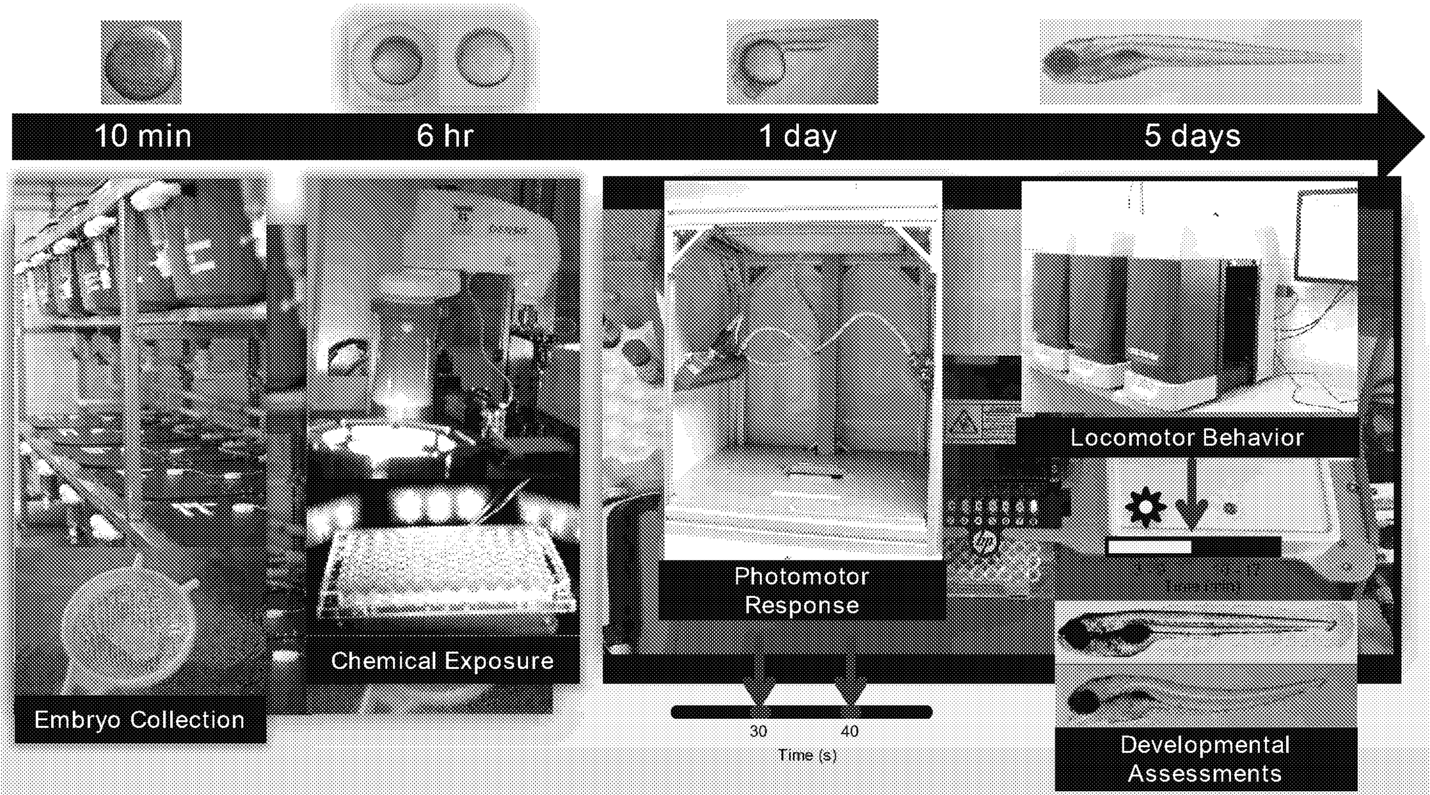
5 day evaluations
- Morphology
- Behavior
- 120 hpf Tissue
- Collection



Static Single Exposure
- Dechorionated
- 6 hpf to 120 hpf
- 8 concentrations
    - 0.001 to 100 µM
- N=32/conc.

24 hour evaluations
- Behavior
- Morphology

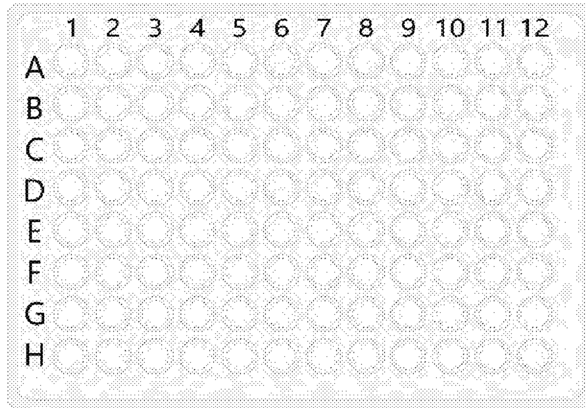
Our HTS Platform



Truong et al. (2014) Toxicol Sci 137: 212-233.

Mandrell, D., Truong, L., et al . 2012. Automated zebrafish chorion removal and single embryo placement: Optimizing throughput of zebrafish developmental toxicity screens. *Journal of Laboratory Automation 17 (1) 66-74.*

Range-finding



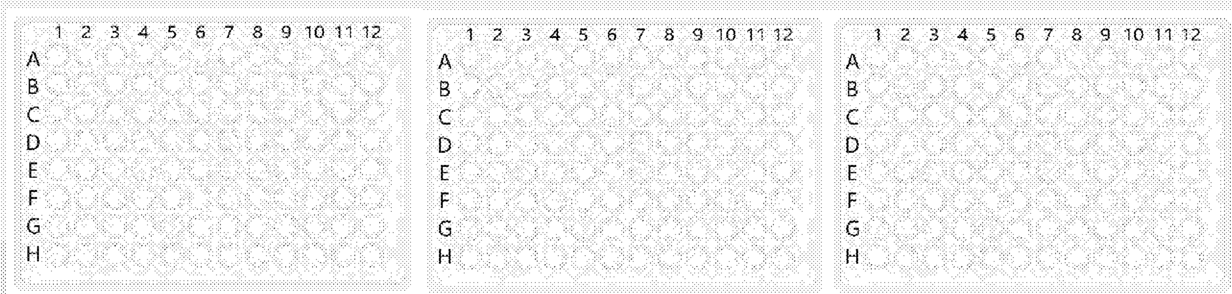
100 uM
74.8
35
16.4
6.45
2.54
1
0

1 chemical per plate
8 concentrations, n = 12

With all 430 chemicals, only conduct morphology assessments up to 5 dpf

<u>Objective:</u> Determine concentrations that cause 0% and 100% bioactivity

Definitive Testing



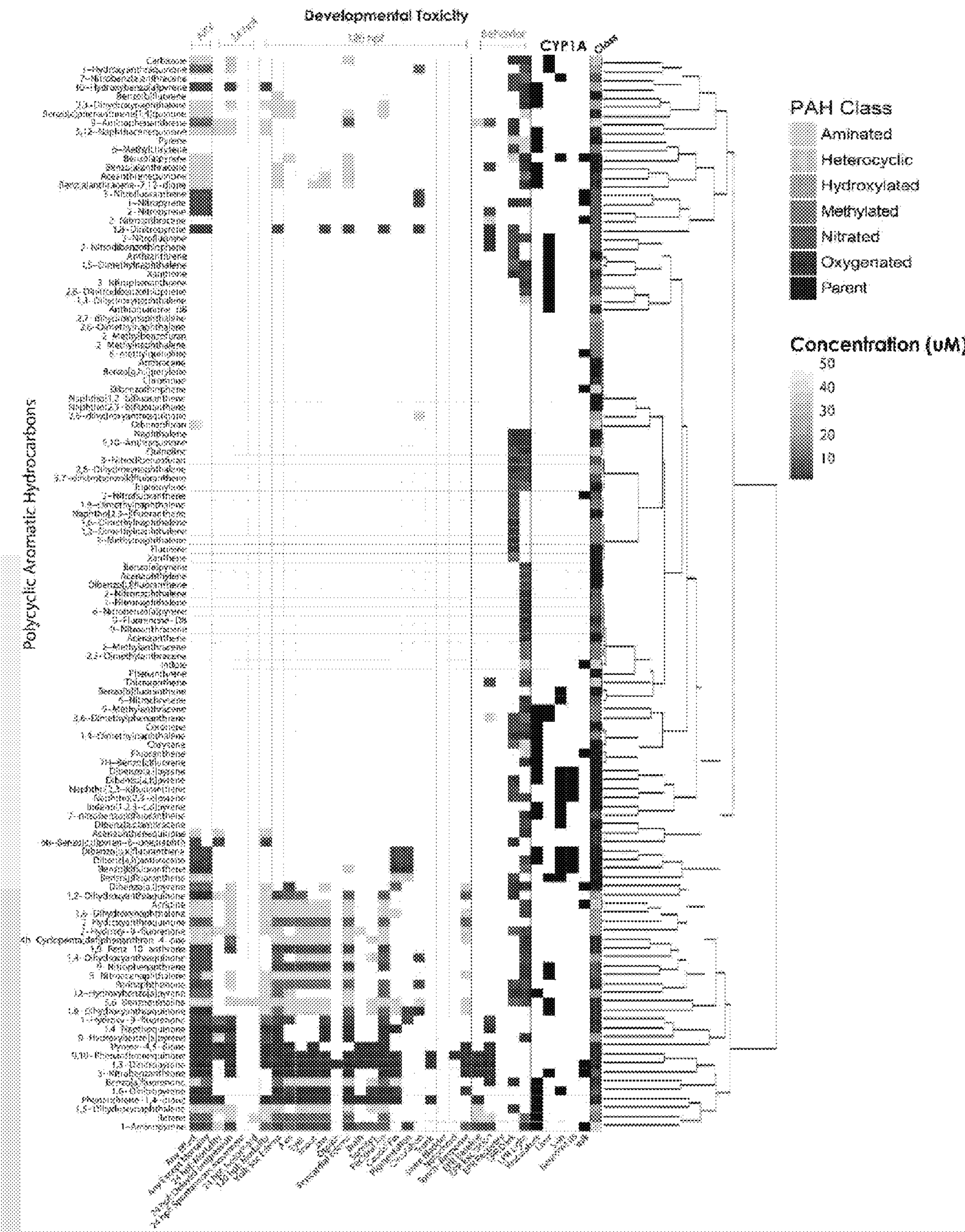
3 replicate plates per chemical
8 concentrations N=32/chemical

With all 430 chemicals, full suite of screening assays through 5 dpf
<u>Objective:</u> Focus in on identifying BMC to generate a well-defined concentration-response curve.

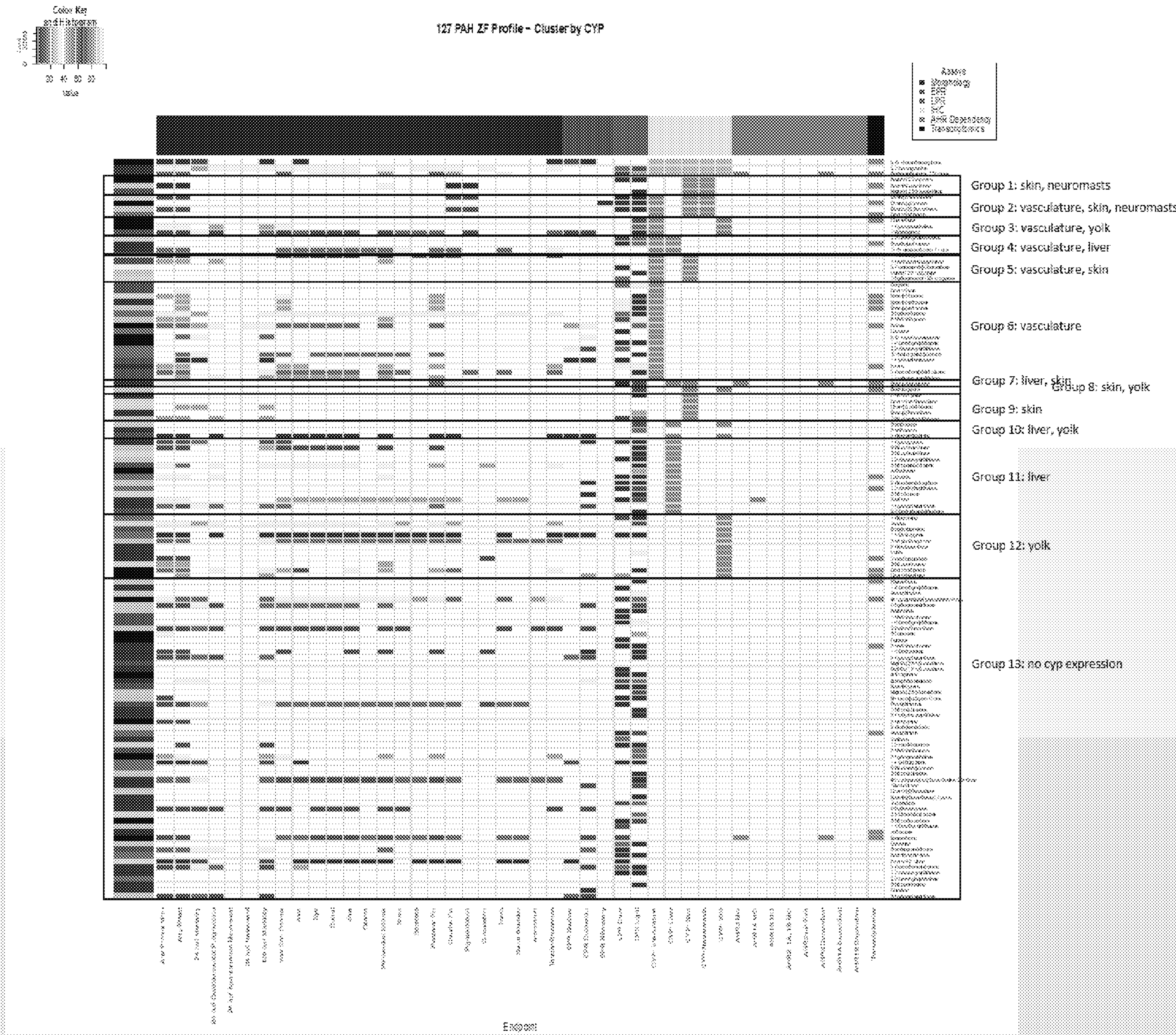
Example of Clustered Response data - PAHs

In this example

- **127 unique compounds**
- **Over two dozen response phenotypes**
- **Possible to discern chemical groupings**

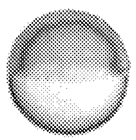


Example of Clustered Response data - PAHs



Global Transcriptomic Analysis of select PFAS compounds

6 hpf 48 hpf 120 hpf



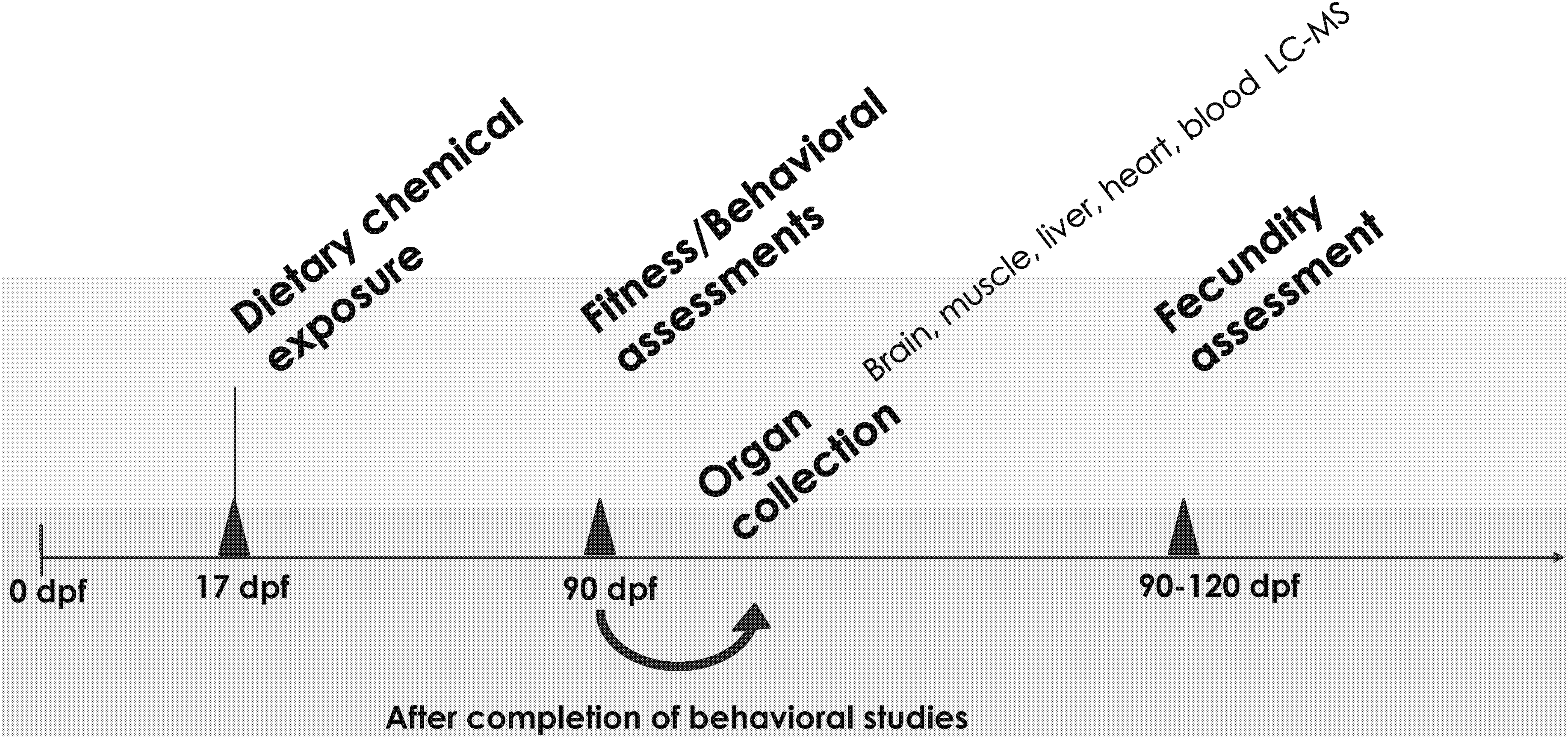
Static Single Exposure
- **Dechorionated**
- **6 hpf to 48 hpf**

48 hour Total RNA Collection
N=4 for each PFAS

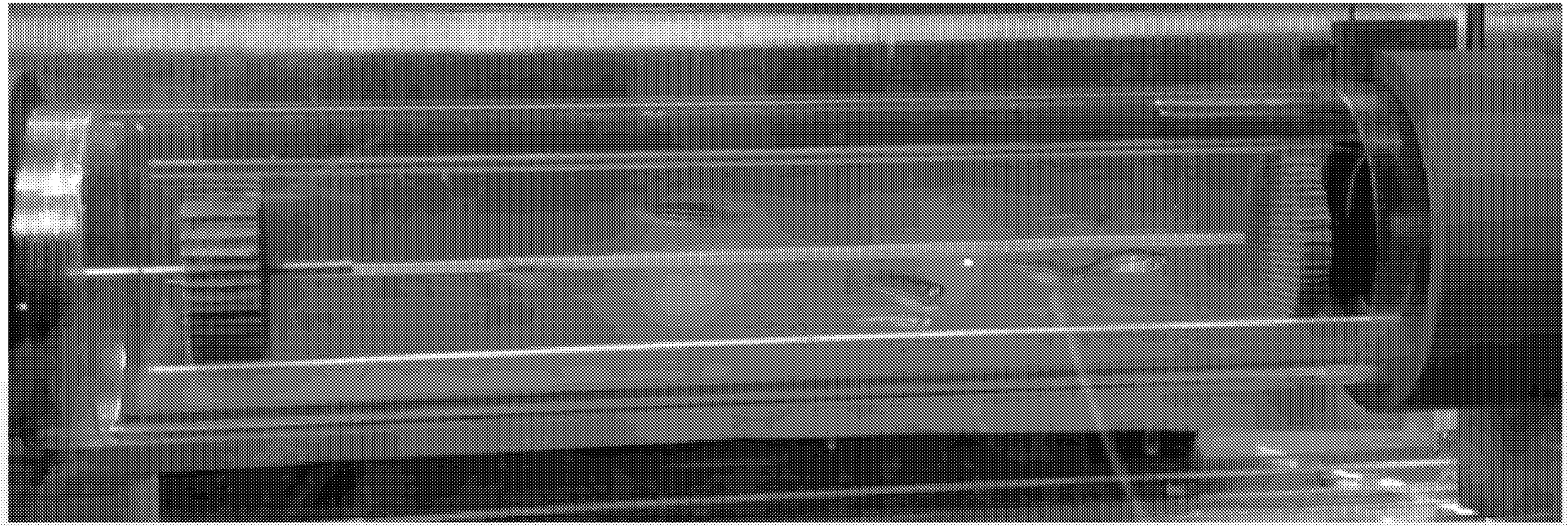
Screen embryos at 24 and 120 hpf for a suite of ~20 morphological endpoints. Use logistic regression modeling of response data to calculate EC_{80}.

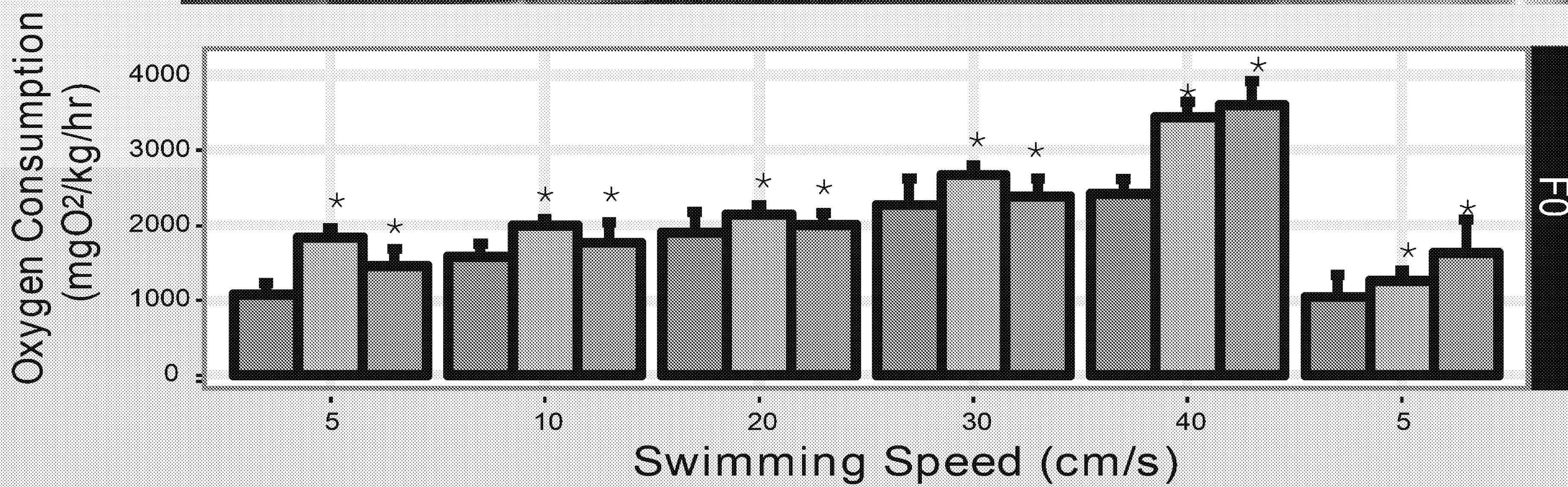
Juvenile Dietary Exposure

Juvenile Dietary Exposure & Organ Body Burden Timeline

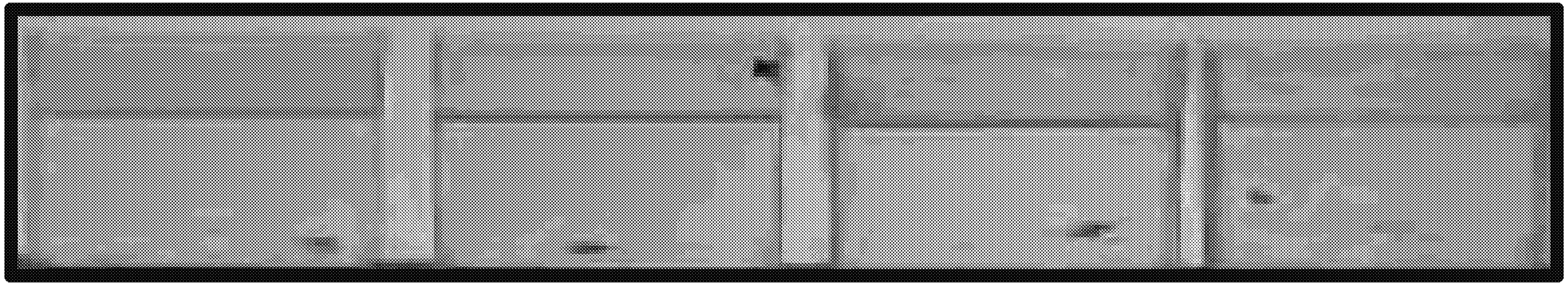


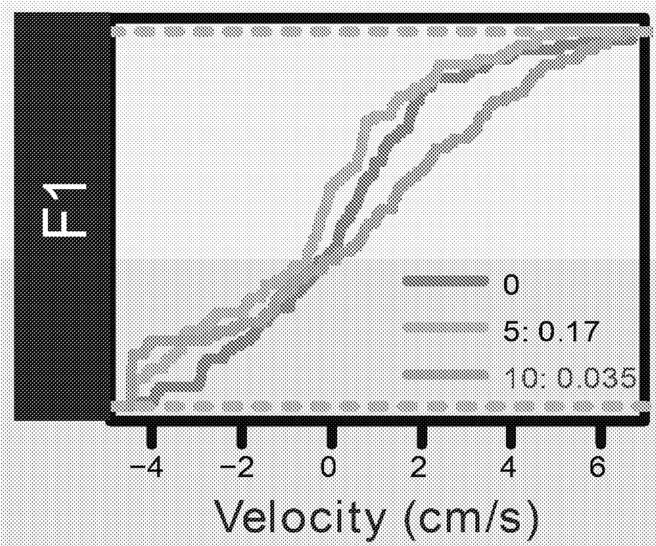
Adult Fitness



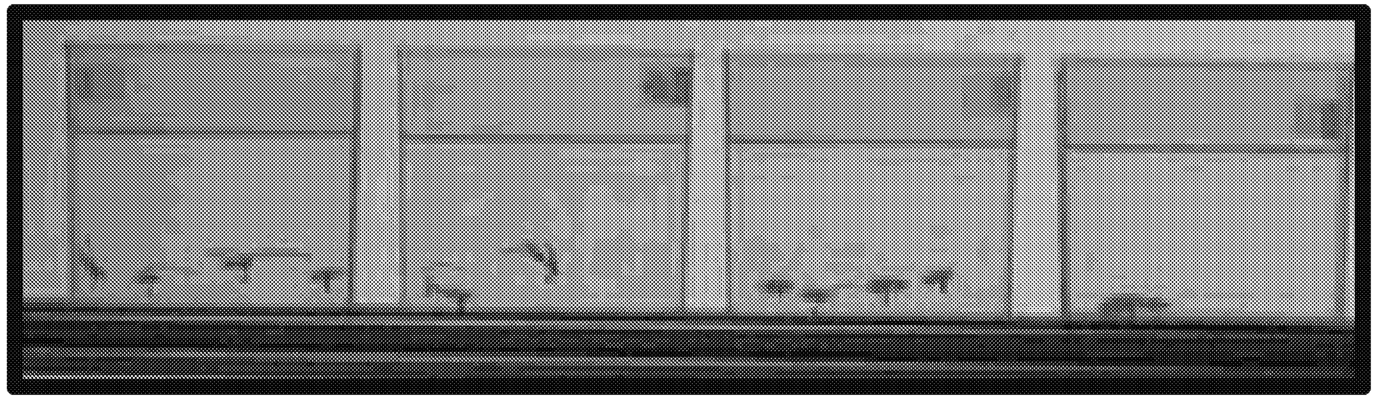


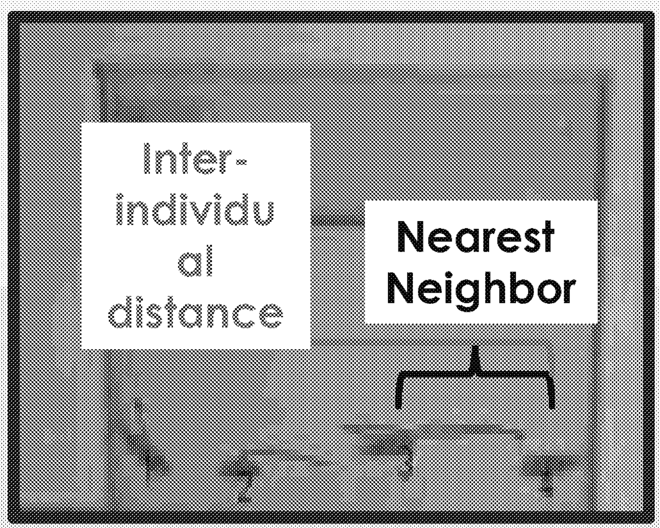
Adult Swimming Activity Over Time

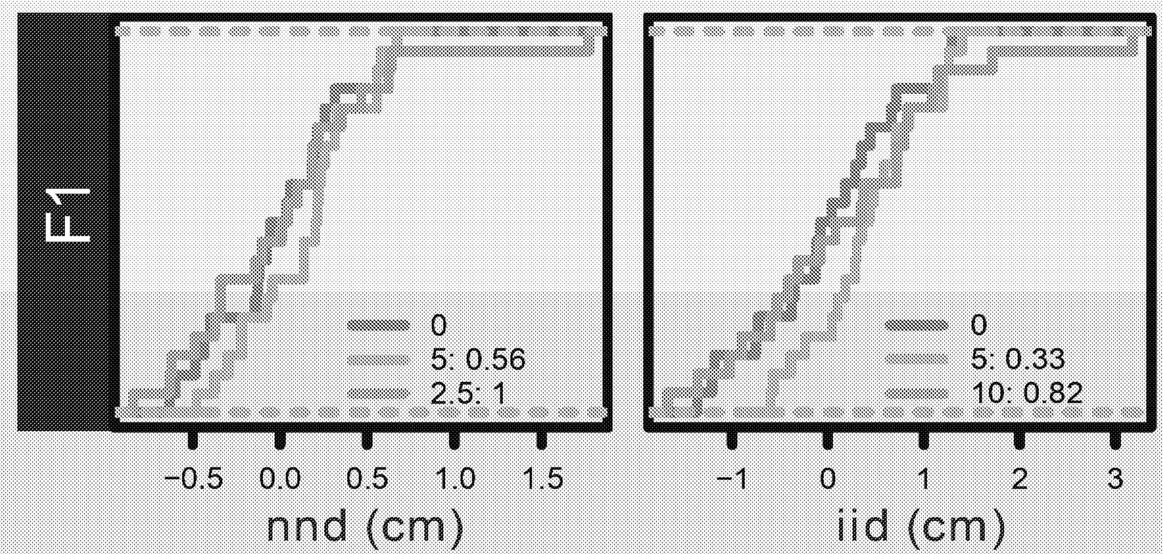




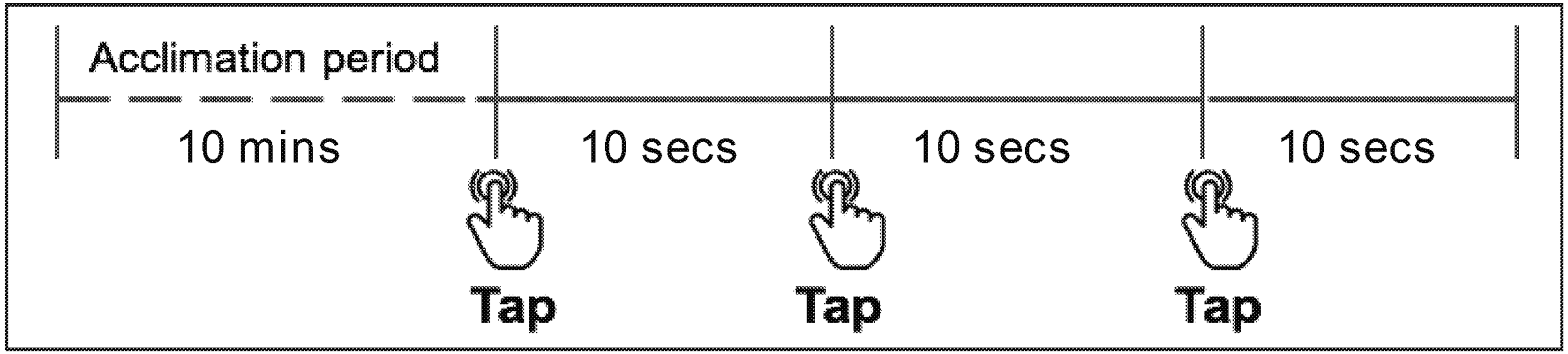
Adult Social Interactions

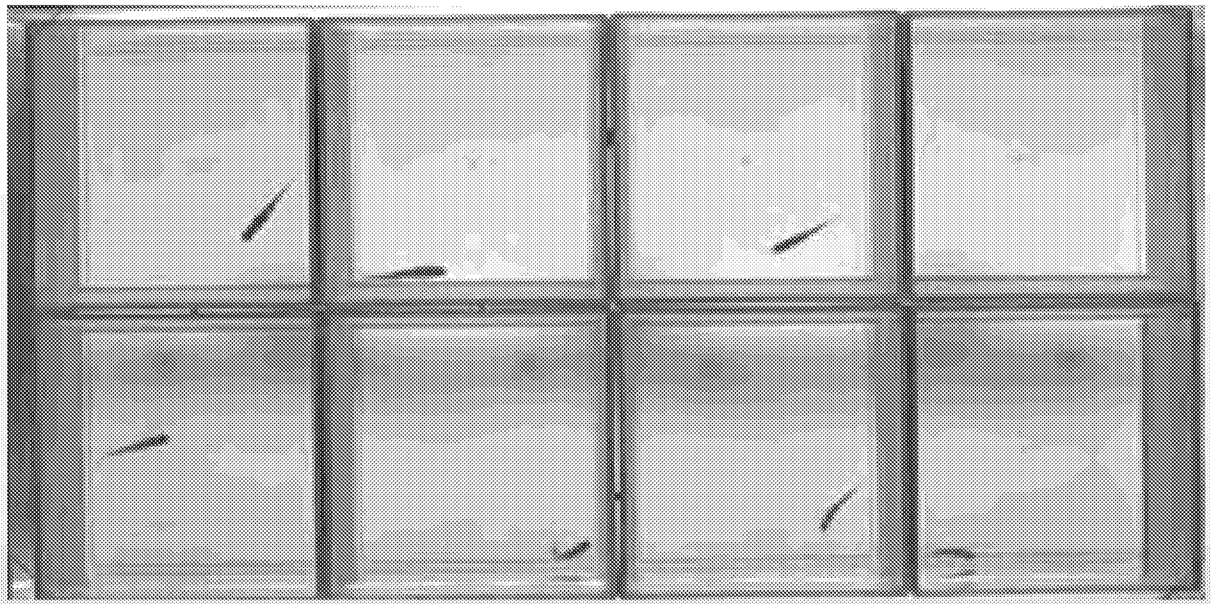


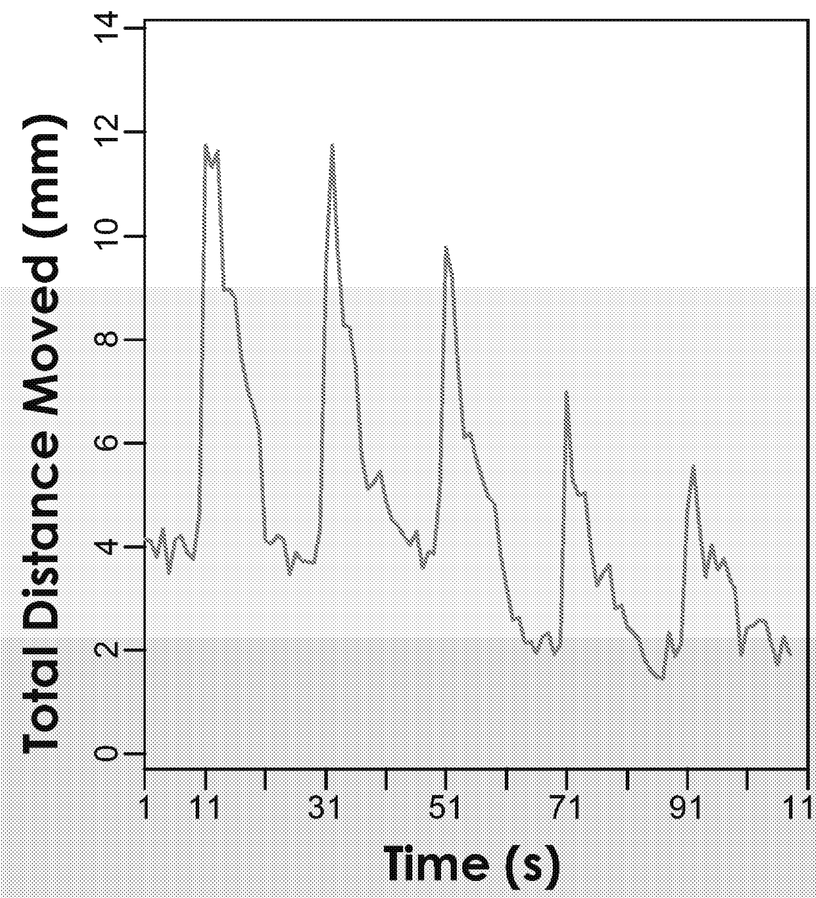




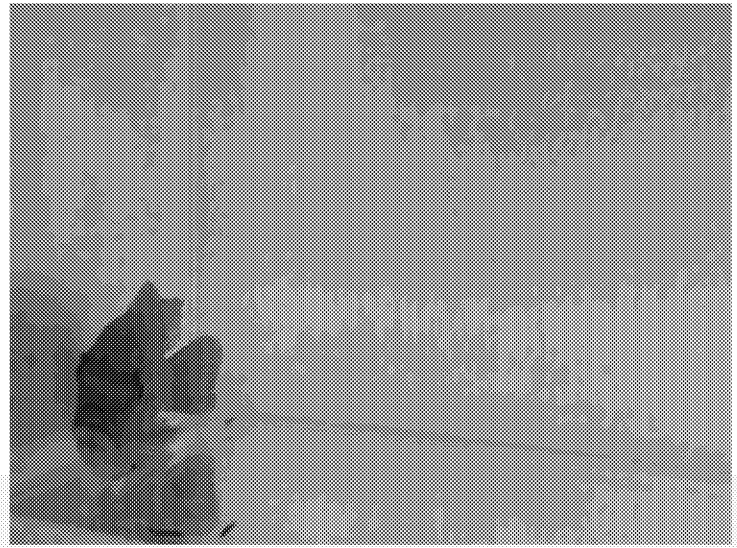
Adult Anxiety

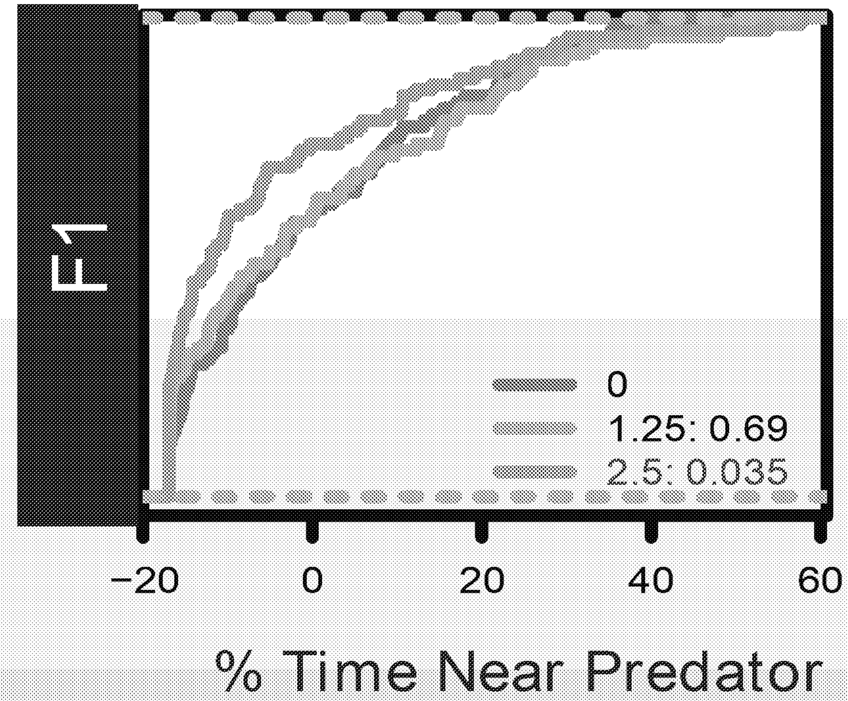




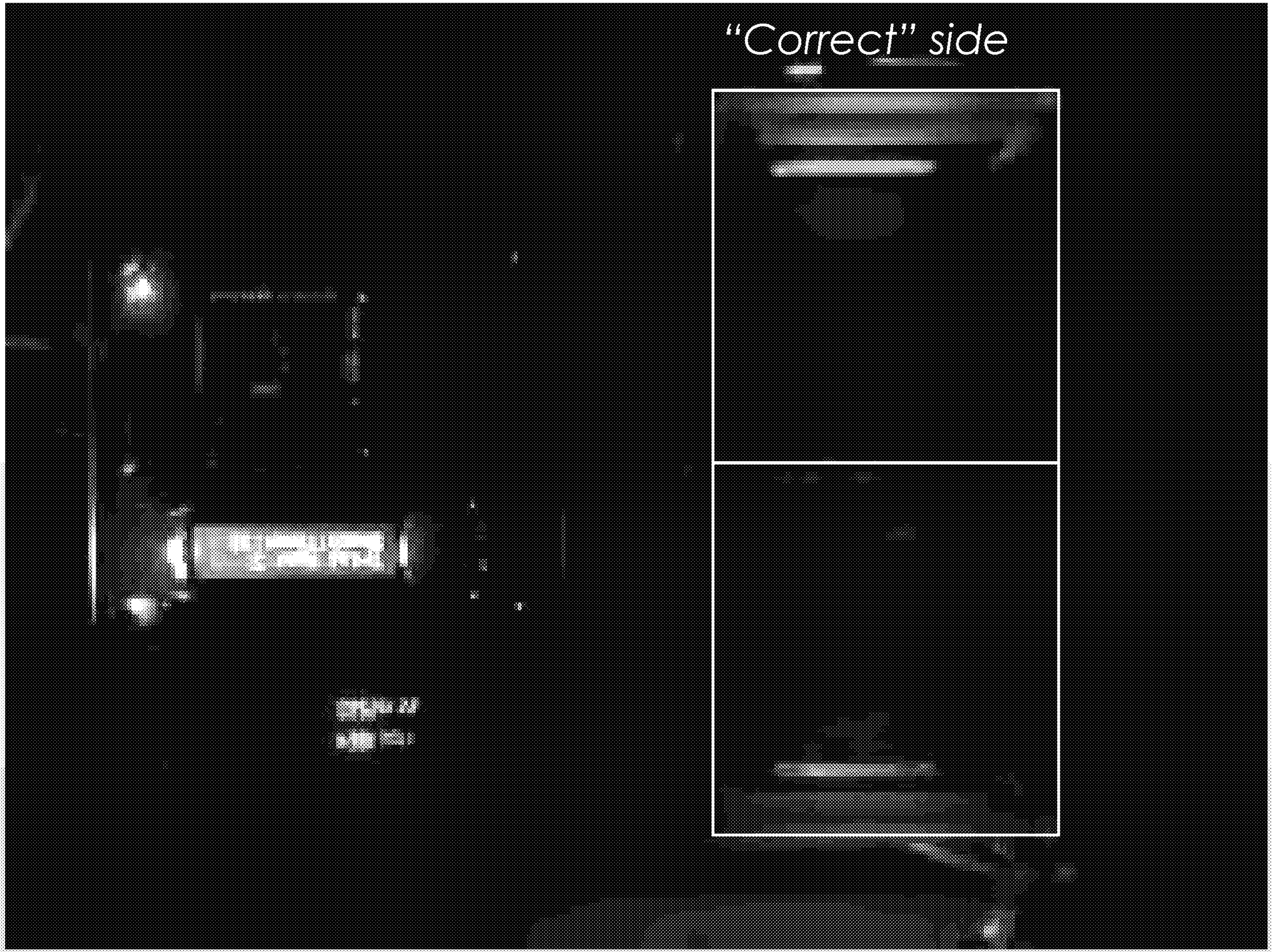


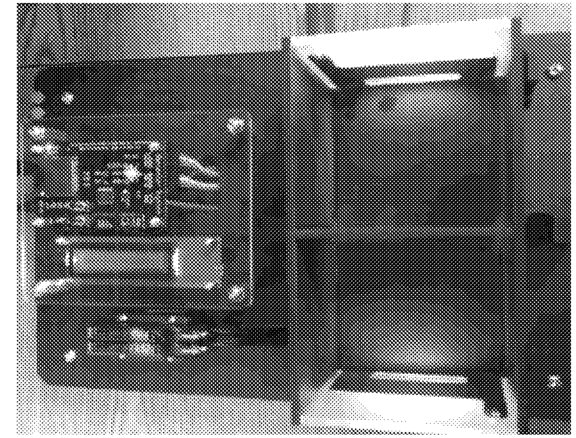
Adult Fear Response





Adult Learning





Chem

B[a]P Conc (uM)	Shocked Time	Time to Correct Side	Time on Correct Side
4	↑*	↑*	↓*

Summary

- Massive data collection effort
- Will require multidisciplinary coordiantion
- Data analysis and sharing crucial for success
- Excited to collaborate with EPA and other partners

Members of this Team

Tanguay Lab

Dr. Lisa Truong
Dr. Michael Simonich
Yvonne Rericha
Jane La Du
Eric Johnson
Carrie Barton
Kim Hayward

Engineering Team

Corwin Perrin
Vance Langer

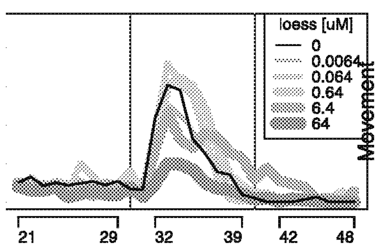
Collaborators

NC State University
David Reif's Group

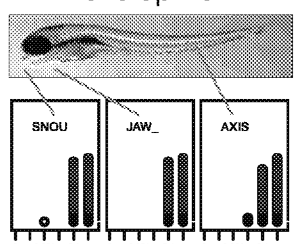
System toxicological approaches to define and predict the toxicity of Per and Polyfluoroalkyl Substances

David M. Reif, PhD
Associate Professor
Bioinformatics Research Center
Center for Human Health & the Environment
Department of Biological Sciences
North Carolina State University
www.reif-lab.org

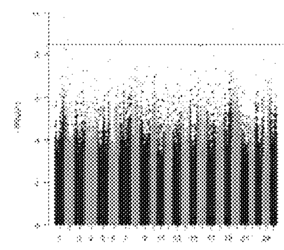
Behavior



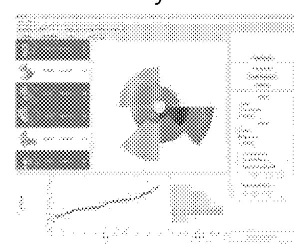
Development



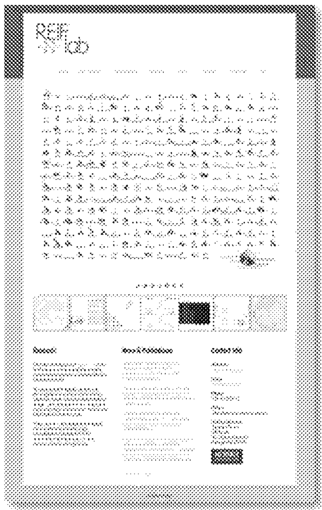
Genetics



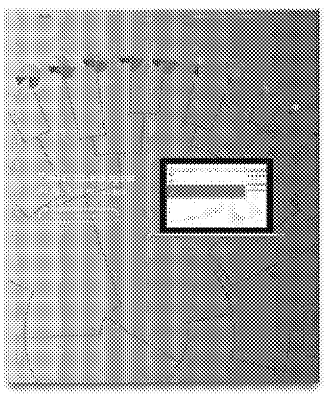
Analytics



Introduction



www.reif-lab.org



www.toxpi.org

Associate Professor,
Department of Biological Sciences (BioSci)

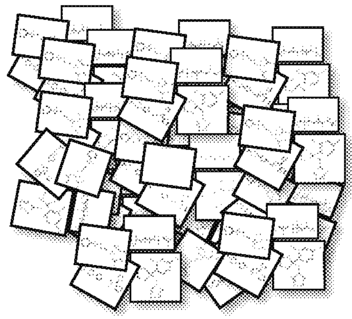
Director,
Bioinformatics Consulting and Service Core (BCSC)

Bioinformatics Team Lead,
Center for Human Health and the Environment (CHHE)

Resident,
Bioinformatics Research Center (BRC)

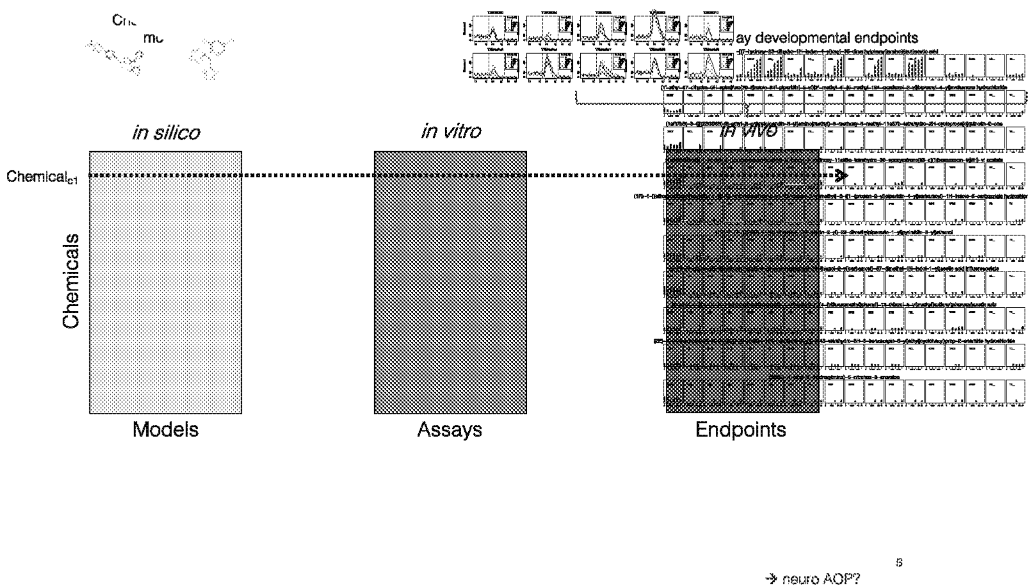
Graduate Training Faculty,
- Bioinformatics, North Carolina State University
- Functional Genomics, North Carolina State University
- Toxicology, North Carolina State University
- Statistics, North Carolina State University
- Genetics, North Carolina State University
- Environmental Sciences and Engineering, UNC Chapel Hill

Research overview:
Data integration to support environmental public health

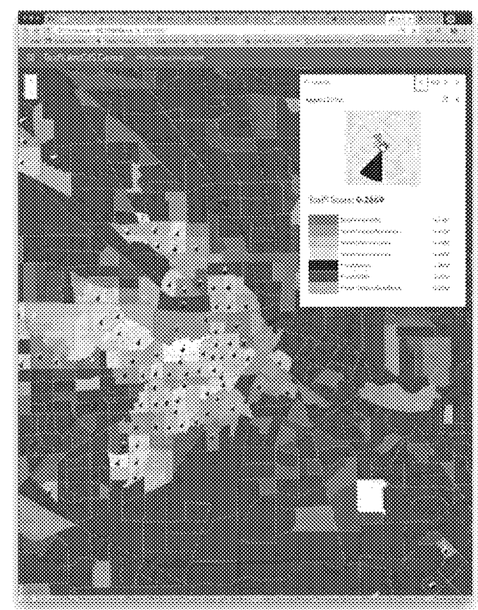


How do we assess hazard for the 100,000s of chemicals currently circulating in our environment?

Data integration across experimental sources

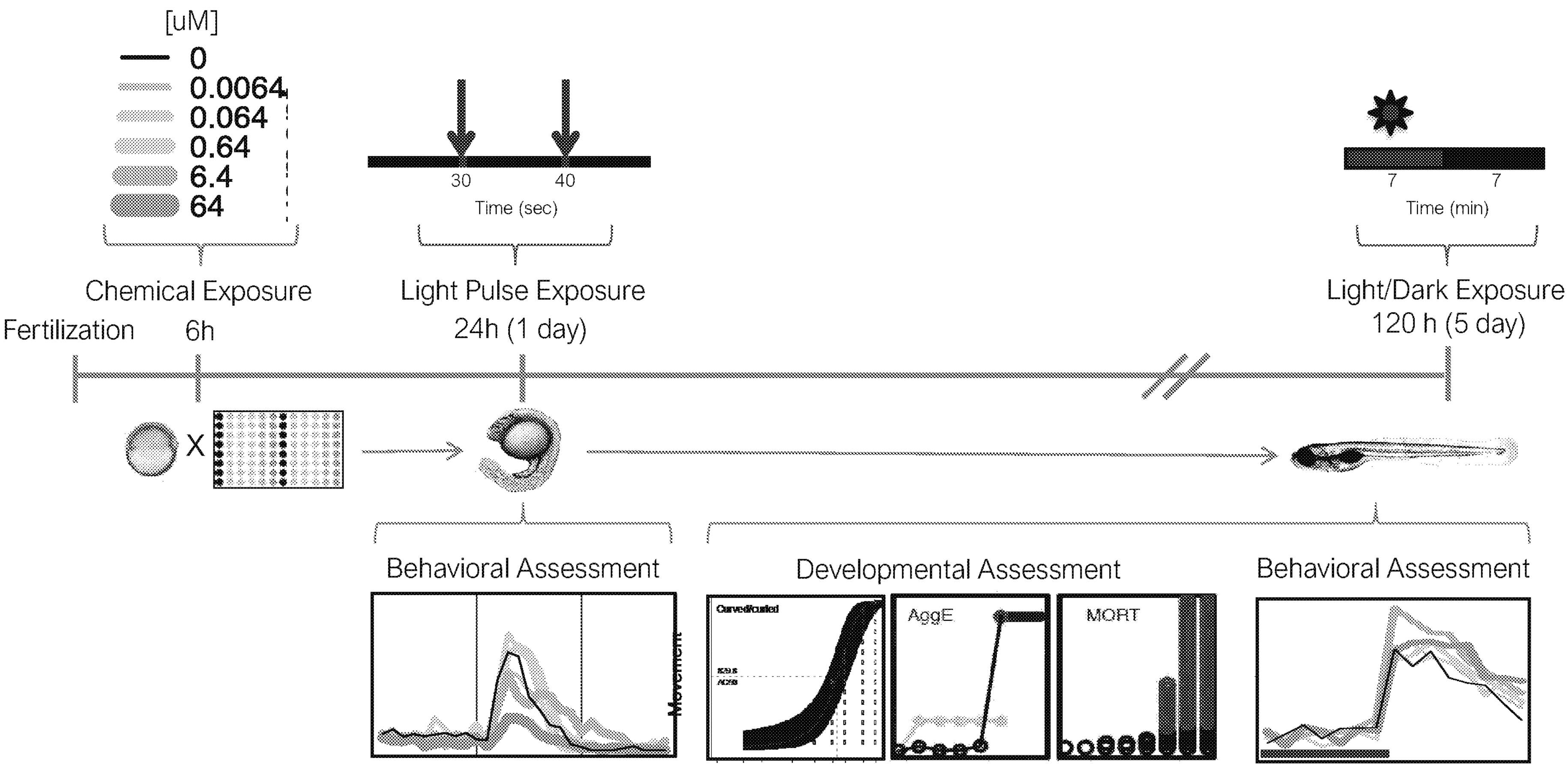


Tools for integrated assessment



ED_005565_00007863-00110

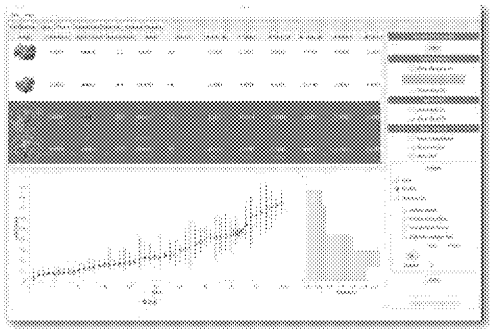
Contributions to this PFAS project: New analytics for assay data



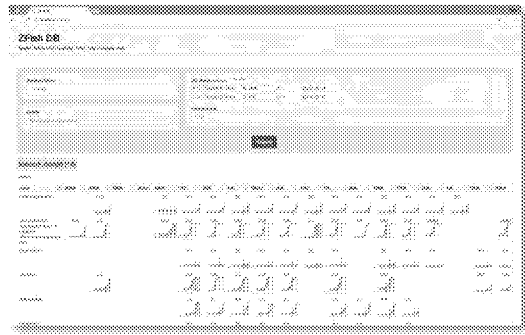
[Knect et al. (2017) *Toxicology and Applied Pharmacology*]
[Zhang et al. (2017) *PLoS One*]
[Zhang et al. (2017) *Toxicology and Applied Pharmacology*]
[Zhang et al. (2016) *Reproductive Toxicology*]
[Reif et al. (2016) *Archives of Toxicology*]
[Truong et al. (2014) *Toxicological Sciences*]

Contributions to this PFAS project: Analysis software and data sharing

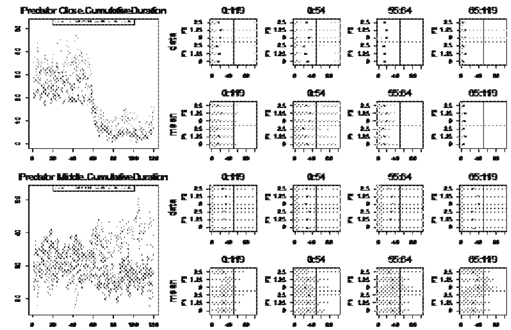
ToxPi GUI for data integration



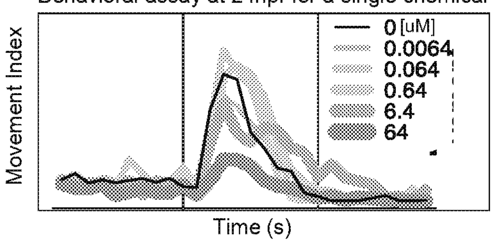
zfish browser for data sharing



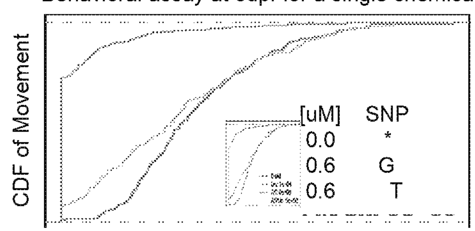
Analysis of complex assay data



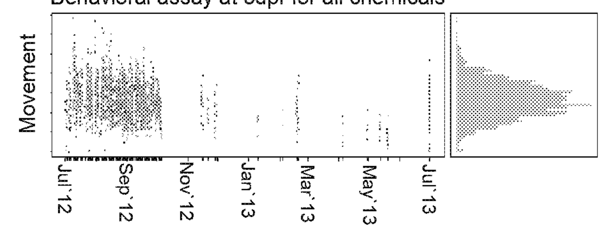
Behavioral assay at 24hpf for a single chemical



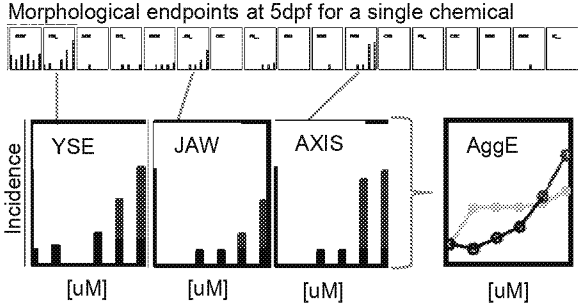
Behavioral assay at 5dpf for a single chemical



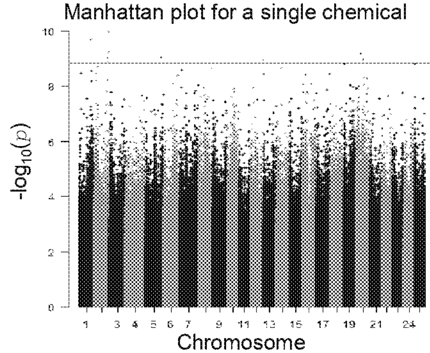
Behavioral assay at 5dpf for all chemicals



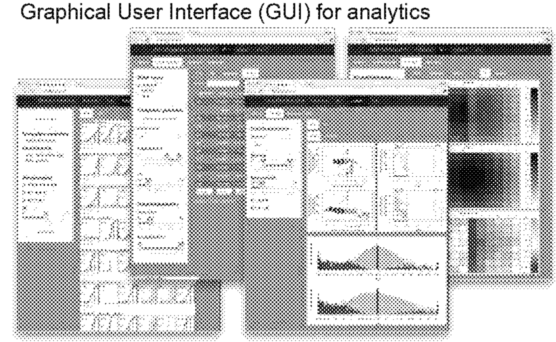
Morphological endpoints at 5dpf for a single chemical



Manhattan plot for a single chemical



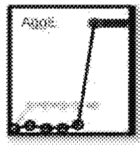
Graphical User Interface (GUI) for analytics

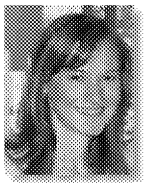


Members

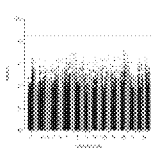
PhD Graduates @ NC State

Guozhu Zhang,
B.S. Mathematics
M.S. Statistics
Ph.D. Bioinformatics
(Graduated 2016)

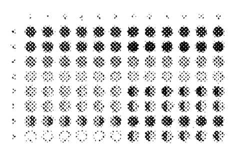




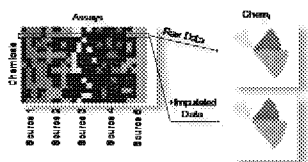
Michele Meisner,
B.A. Statistics
M.S. Statistics
Ph.D. Bioinformatics
(Graduated 2017)



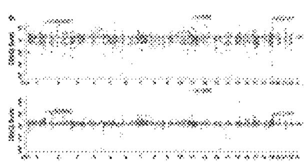
Kyle Roell,
B.S.E. Computer Science
M.S. Statistics
Ph.D. Bioinformatics
(Graduated 2018)



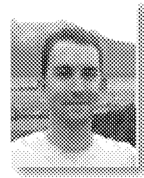
Kim To,
B.S. Statistics
M.S. Statistics
Ph.D. Bioinformatics
(Graduated 2019)



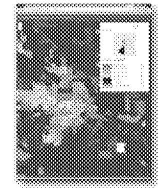
Marissa Kosnik,
B.S. Biochemistry
B.A. Chemistry
Ph.D. Toxicology
(Graduated 2019)

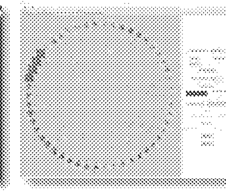


Current Lab Members (in order of appearance)

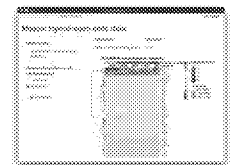


Skylar Marvel,
B.S. Electrical Engineering
M.S. Biomedical Engineering
M.S. Electrical Engineering
Ph.D. Bioinformatics
(Research Associate)

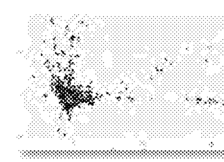




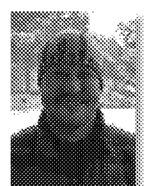
Aldo Carmona-Baez,
B.S. Genomic Sciences
(Ph.D. Student, Genetics)
joint with Reade Roberts



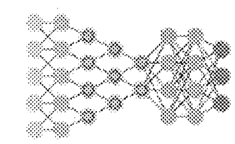
Dylan Wallis,
B.S. Cell & Molecular Biology
B.S. Chemistry
(Ph.D. Student, Toxicology)



Preethi Thunga,
B.S. Biotechnology
(Ph.D. Student, Bioinformatics)

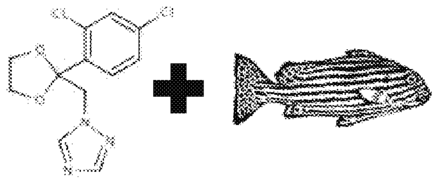


Adrian Green,
B.S. Microbiology
M.S. Pharmacology & Toxicology
Ph.D. Toxicology
(Postdoc)



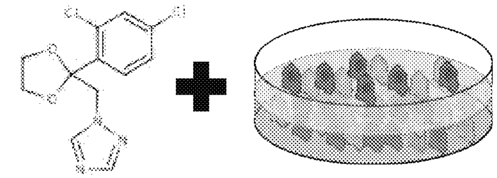
FIN

Data Integration:
Zebrafish HTS generates data complementary to *in vitro* systems

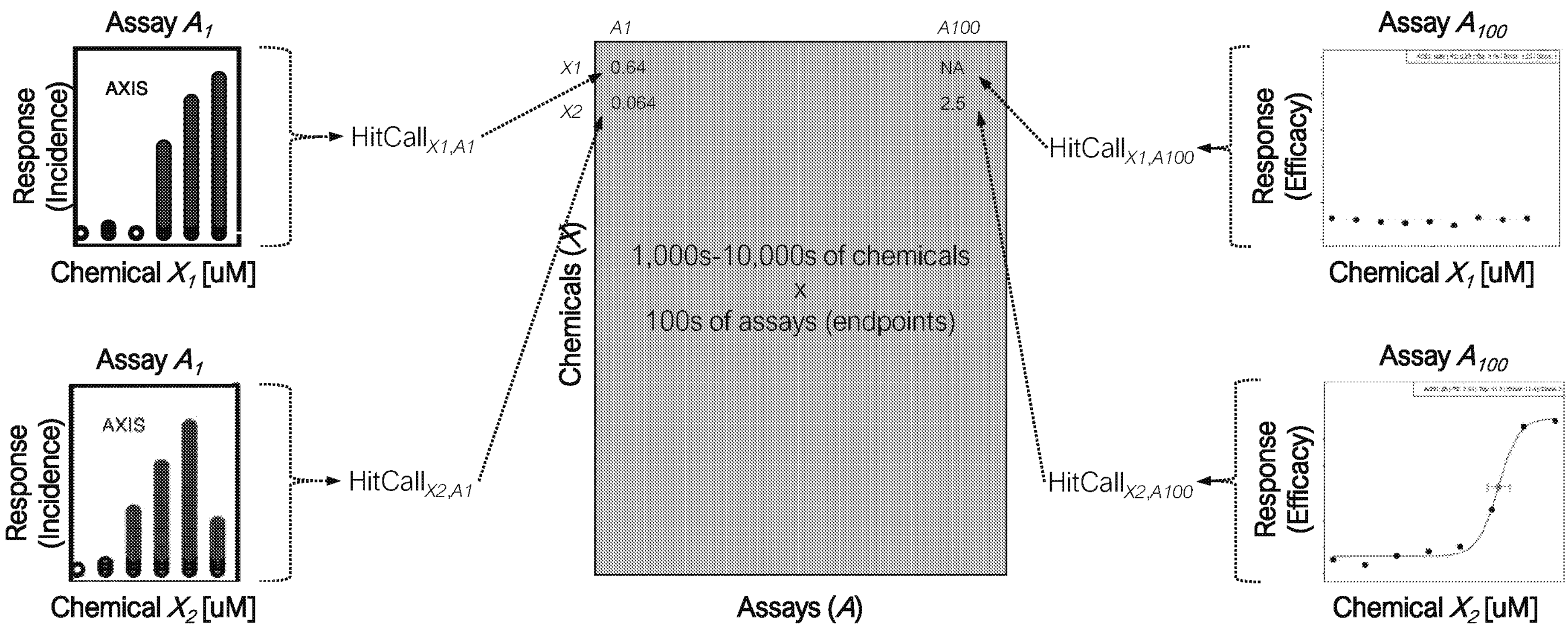


zebrafish assay systems

Chemicals (*X*) are tested in concentration-response mode in all assays (*A*) to generate massive Chemical-Assay data.



***In vitro* assay systems**

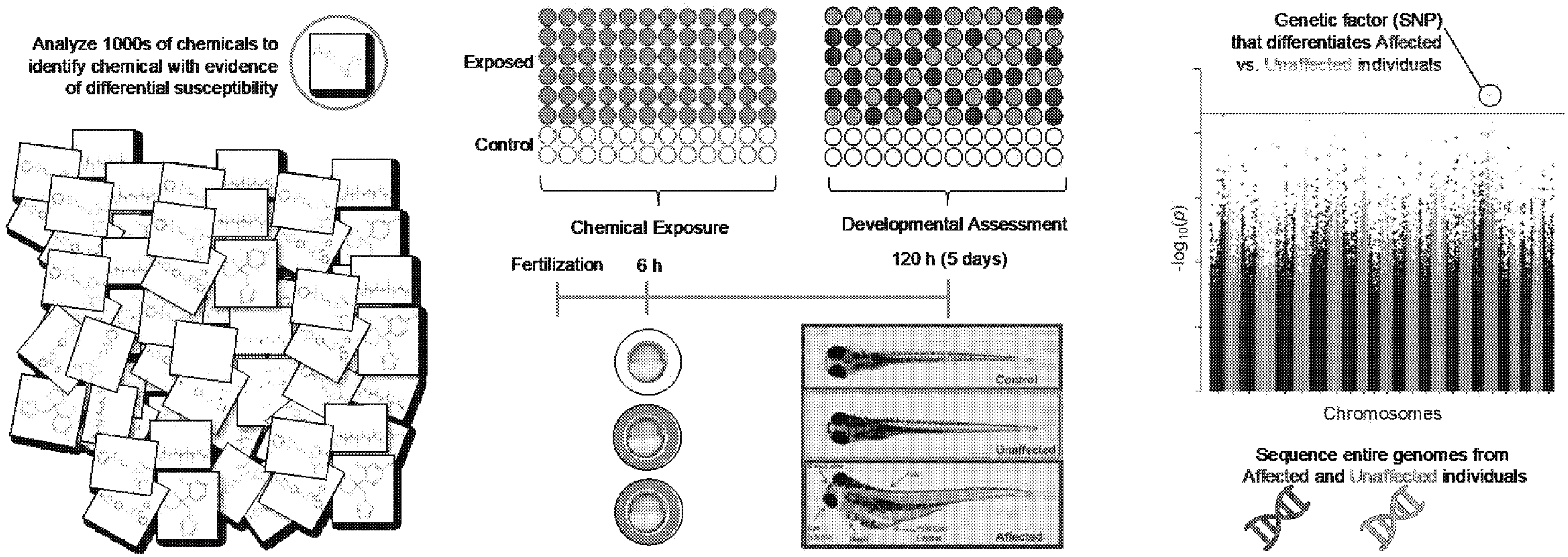


Approach: Does GxE play a role in why the same chemical exposures elicit differential susceptibility across individuals?

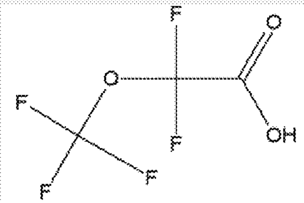
Look for a signal of population variability in HTS data,

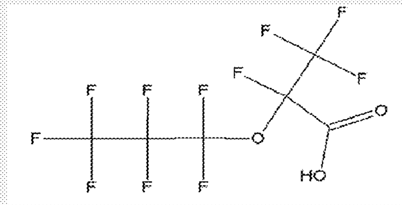
then identify individuals with differential response ( ) to an identical exposure ( ),

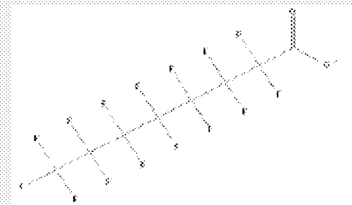
then search the genome to highlight GxE effects.

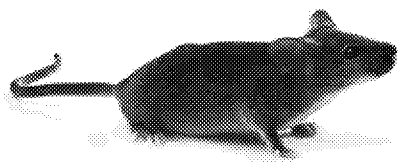


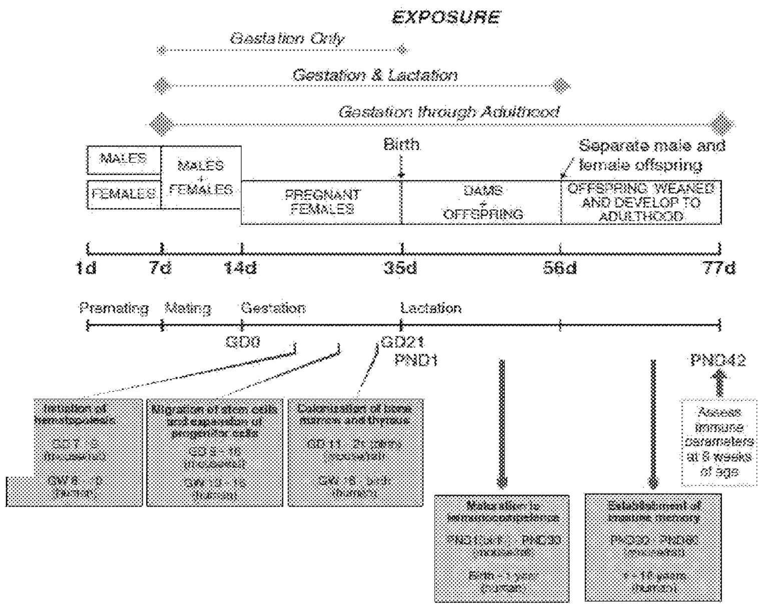
[Balik-Meisner et al. (2018) *Environmental Health Perspectives*]

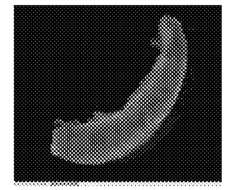


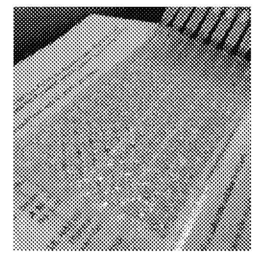


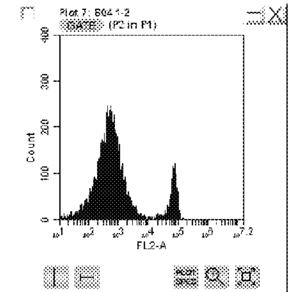


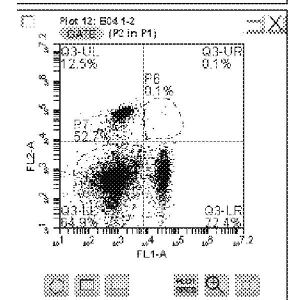










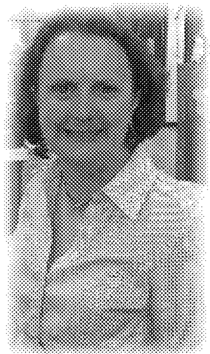


Capabilities and contributions of the DeWitt Lab at East Carolina University

Department of Pharmacology and Toxicology, Brody School of Medicine, ECU

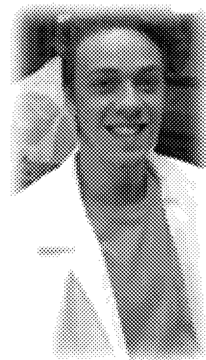
DeWitt Lab people

Qing Hu
Research specialist



Dr. Tracey Woodlief
Postdoc

Emma Baker
(tentative) MS student



Mark Ibrahim
Undergraduate student

Jeff Ayala
Undergraduate student

Jasmine Clark
Undergraduate student

One new doctoral student

Javier Limon
High school student

John Mallett
High school student

Why evaluate the immune system folowing PFAS exposure?

PFOA and PFOS are presumed to be immune hazards to humans.

National Toxicology Program
U.S. Department of Health and Human Services

SYSTEMATIC REVIEW OF
IMMUNOTOXICITY ASSOCIATED WITH EXPOSURE TO
PERFLUOROOCTANOIC ACID (PFOA) OR PERFLUOROOCTANE
SULFONATE (PFOS)

June 6, 2016

PFOA and PFOS suppress antigen-specific antibody responses in experimental models (high level of evidence) and humans (moderate level of evidence).

Other immune effects supporting this weight-of-evidence classification:

- Increased hypersensitivity-related outcomes.
- Suppression of innate immune responses (i.e., NK cell function).
- Alterations in disease resistance/infectious disease outcomes.
- Findings of autoimmunity.

Why evaluate the immune system following PFAS exposure?

Human benchmark dose levels for suppression of diphtheria and tetanus vaccines were ~1.3 ng/mL serum for PFOS and ~0.3 ng/mL for PFOA at a benchmark dose response of 5%.

As a comparison, median PFOS and PFOA serum concentrations from NHANES participants (2015-2016 sample years) were 5 ng/mL and 2 ng/mL, respectively.

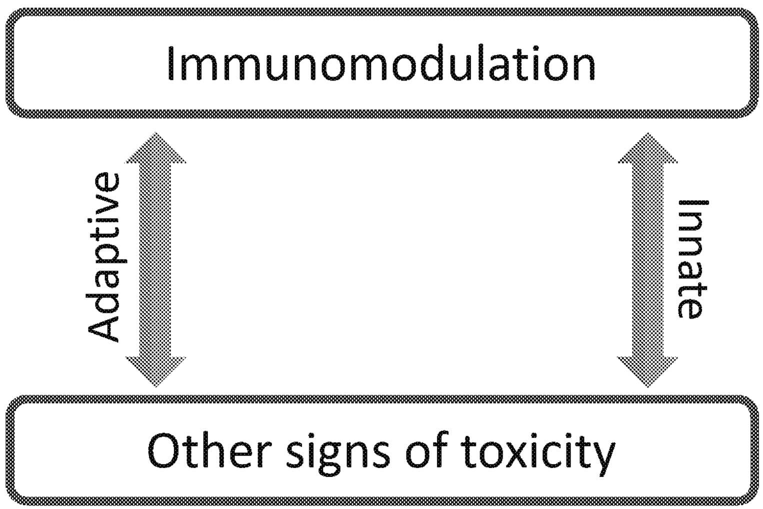
Human equivalent dose (HED) for PFOA-induced immune suppression in mice, for example, is 0.0053 mg/kg/day.

As a comparison, this was the same HED for the critical effect (developmental toxicity) chosen by the US EPA to calculate the reference dose for PFOA.

The immune system is sensitive to PFAS exposure!

Data from: Grandjean and Budtz-Jorgensen. 2013. Environmental Health; US EPA, 2016.

DeWitt Lab approaches to understudied PFAS



Production of essential ***descriptive*** data:

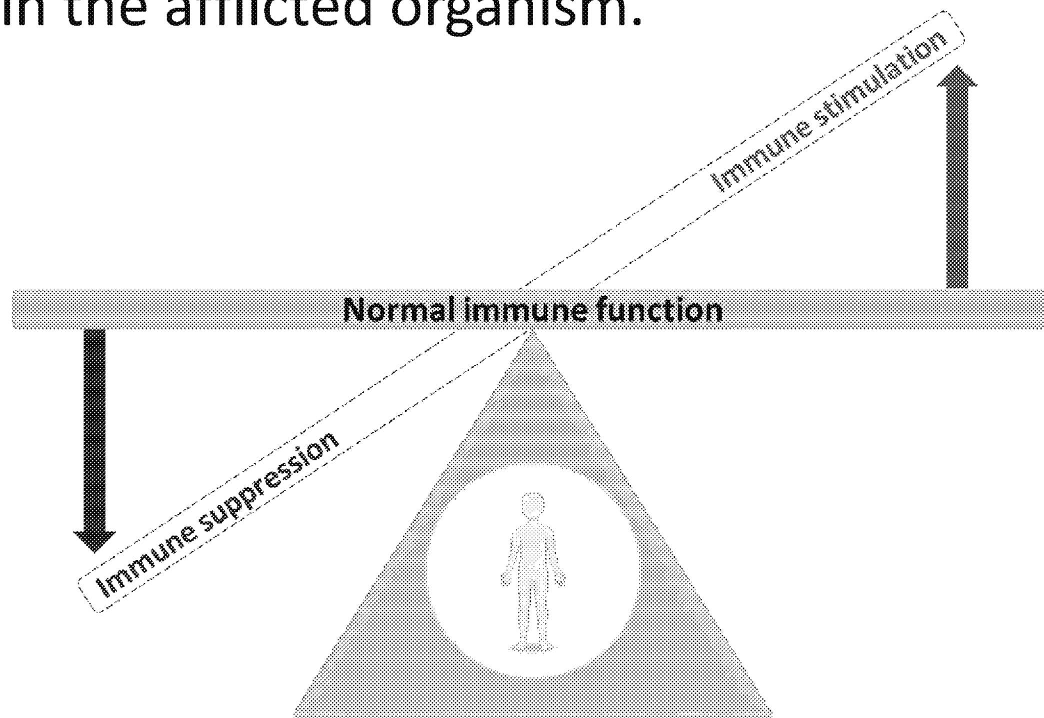
- Dose-response data for basic toxicological endpoints
- Dose-response data for immunotoxicological endpoints
- Functional readouts translatable to human health
- Cellular readouts to support additional mechanistic investigations

These descriptive data are critical for decision-makers.

DeWitt Lab approaches to understudied PFAS

Our focus is on functional immune suppression:
Reduced ability of the immune system to respond to a challenge from a level considered normal, regardless of whether clinical disease results in the afflicted organism.

Evaluation of functional immune suppression can provide accurate information on risks to the immune system from chemical exposures.

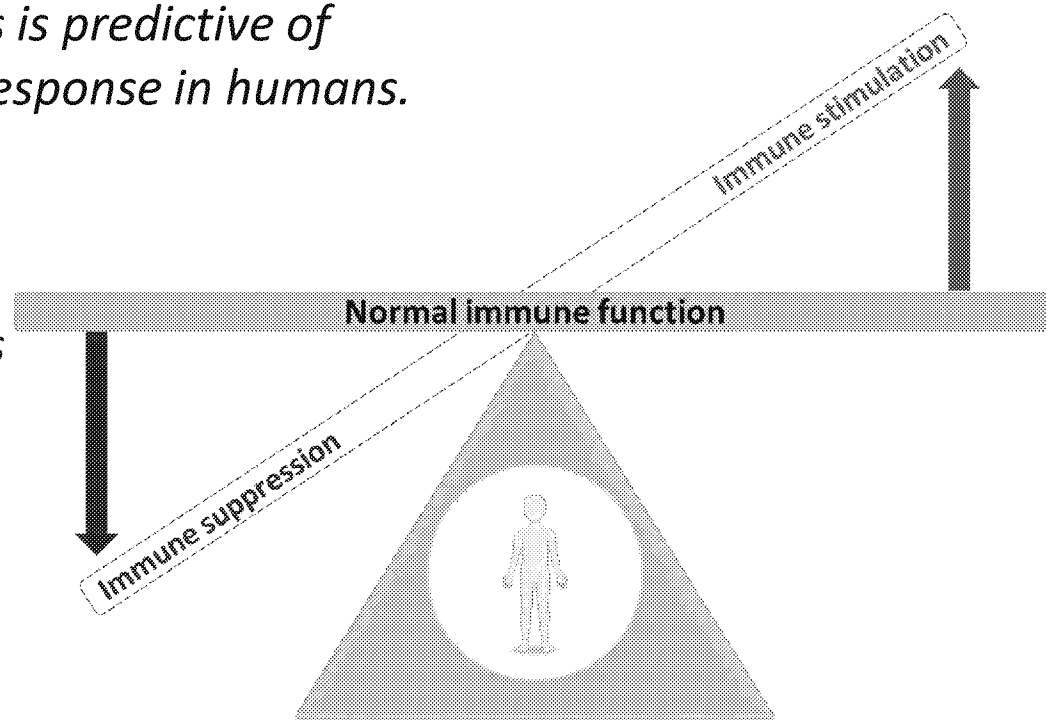


Definition from DeWitt et al. 2016. *Current Protocols in Toxicology*.

DeWitt Lab approaches to understudied PFAS

We assess immune function:

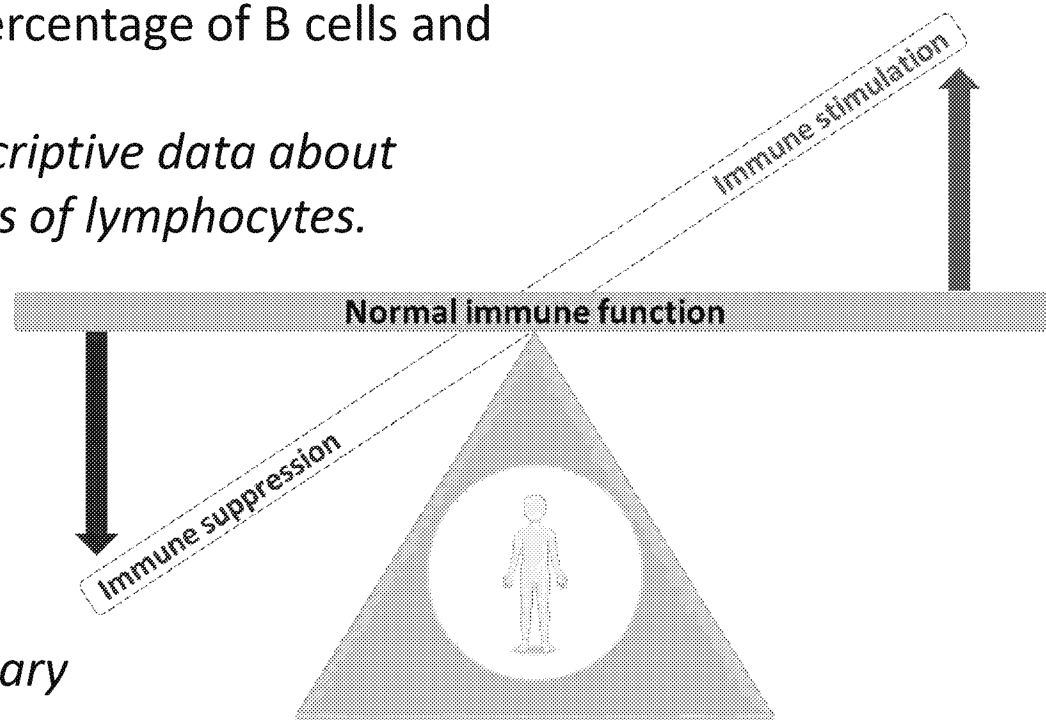
- Adaptive immunity: T cell-dependent antibody response (TDAR); analogous to human vaccine response.
    - *Suppression in rodent models is predictive of likelihood of suppression of response in humans.*
- Innate immunity: Natural killer (NK) cell cytotoxicity; part of the cancer cell surveillance system.
    - *Suppression in rodent models is predictive of likelihood of suppression of response in humans.*
- These responses cover multiple arms of immune function.



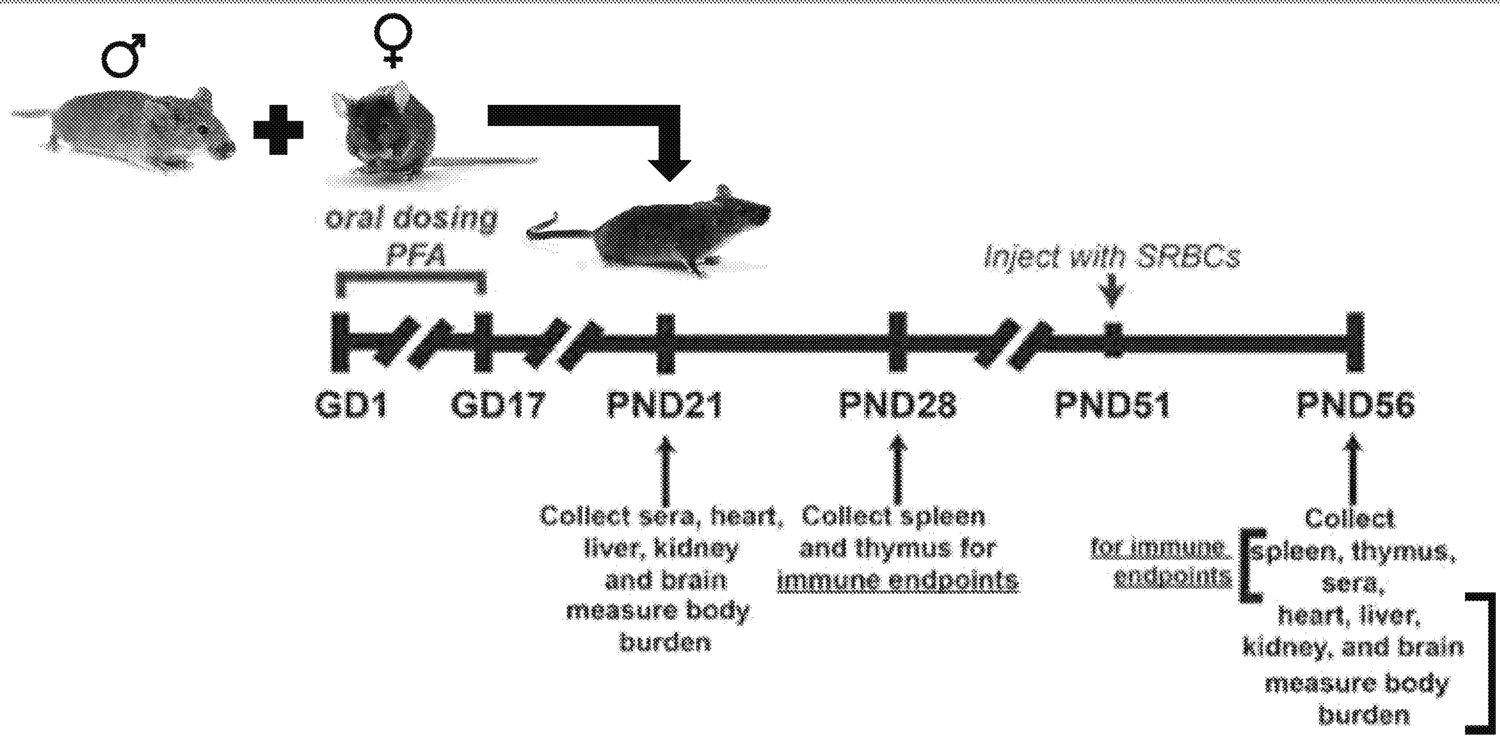
DeWitt Lab approaches to understudied PFAS

We also describe other observations:

- Immune organ endpoints: Spleen and thymus weights and total number of cells within each organ.
- Immunophenotype: Percentage of T cell subpopulations in the thymus and spleen and percentage of B cells and NK cells in the spleen.
    - *These provide important descriptive data about potential impacts on numbers of lymphocytes.*
- Other endpoints: Body weight, general appearance, litter size and weight, sex ratio, developmental markers, organ weights, and more.
    - *These provide important descriptive data about primary and secondary toxicities.*



DeWitt Lab approach to this project



For this project:

- Immune functional data
- Immune observational data
- Descriptive developmental data
- Descriptive basic toxicological data

These data are translationally-relevant and appropriate for deriving health-based guidance values for protection of human health.

Additional work on PFAS

NORTH CAROLINA
PFAS
Testing Network

Immunotoxicity of understudied PFAS in adult rodent models.
Similar endpoints as for current project, but following adult exposures.

Other related work:

- Metabolic dysfunction as an underlying mechanism of PFAS-induced immunotoxicity. *Postdoctoral scholar research side-project. Grant proposal to be submitted 9/27/19.*
- Effects of PFAS on B and T cell signaling in vitro. *Grant proposal to be submitted 9/20/19*.

Thank you and I welcome your questions!

Oregon State University

PFAS Analytical Support

EPA National Priorities: Per- and Polyfluoroalkyl Substances
Grant Kickoff Meeting, Thursday September 5 2019

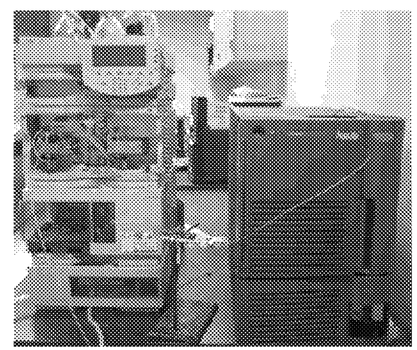
Jennifer Field and Dunping Cao
Department of Environmental & Molecular Toxicology

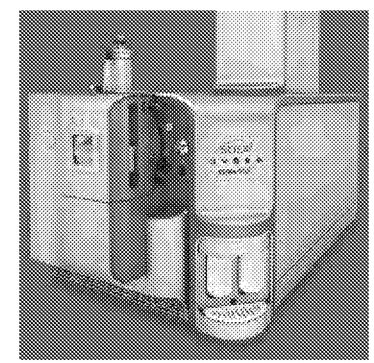
Field Lab: PFAS Analysis

Oregon State University

- 22+ years experience in PFAS analysis
- Current capability
  - 400+ PFAS by LC-MS/MS (Higgins collaborator)[1]
  - 21 volatile PFAS by GC-MS (Simonich, collaborator)[2]
- Three LC-MS/MS equipped for large volume injection of water, solvents, and extracts (biota)
- LC-QToF for suspect screening and non-target analysis (Higgins, collaborator)
- Orthogonal chromatography captures anionic, zwitterionic, cationic PFAS (no SPE)[3]
  - ng/L quantification limits up to 20,000 ng/L
  - negative and positive mode
- Focus on quality control (QSM)

[1]Barzen-Hanson et al. 2017 ES&T;[2]Rewerts et al., 2018 ES&T;[3]Backe et al.2013





Role on Project

Oregon State University

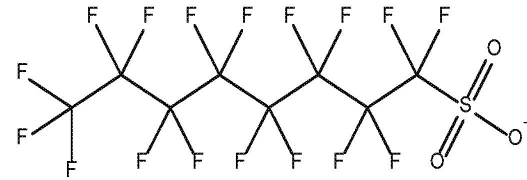
Application of existing LC-MS/MS methods for PFAS in dosing solutions and zebrafish and mouse tissue

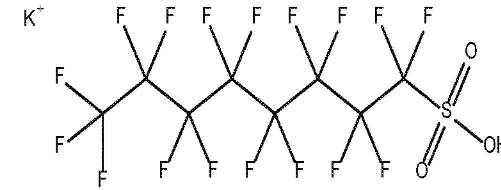
Adaptation of methods for new PFAS (LC-MS/MS and GC-MS)

Quality control of experimental systems (background levels in dosing solution matrix, 96 well plates, zebrafish, mouse exposure materials)

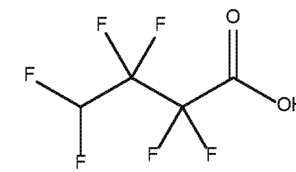
PFAS Standards/Reference Material

- OSU standards
  - Wellington Laboratories ~46 ‘native’ standards
    - Some made because of OSU discovery[1]
  - Fewer stable isotope (^{13}C) labeled standards
  - Second stable isotope (^{13}C) for two PFAS (PFOS and PFOA)

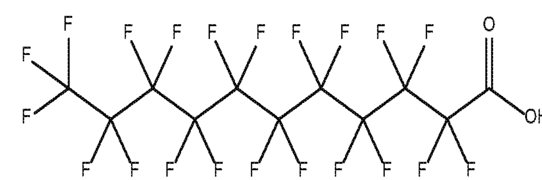


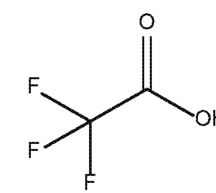


- EPA Library
  - 73 priority PFAS
    - 35% (26) can be analyzed by LC-MS/MS
    - 64% (47) can only be analyzed by GC-MS/MS
  - H^+ and salt (NH_4^+ and K^+) forms for select PFSAs and only H^+ for PFCAs
  - Priority contains many that have standards and some that don’t
  - PFAS with standards fall into low priority but are found in water!
  - Many in GC-MS list have no standard, method, or data in literature (a real unique opportunity but also an analytical challenge)



[1]Place et al. 2012 ES&T;Barzen-Hansen et al. 2017 ES&T; Barzen-Hansen et al. 2015 ES&T





Data Quality Tiers

Oregon State University

- Quantitative
  - Native standard and stable isotope labeled standard available
- Semi-quantitative
  - Native standard but no stable isotope labeled standard
- Screen
  - Reference material (EPA library) available but no native or stable isotope labeled standard

Quality Control

Oregon State University

- Check all materials in zebrafish and mouse exposure studies for background (LC-QToF) for 400+ PFAS
- 3rd party reference to ensure accuracy for 'core' PFAS
  - PFCAs and PFSAs
- Solvent and extraction blanks in all analytical sequences
- Mass balance approach on 96 well plates (check for loss to plates)
- Adhere to QSM Table B-15 on LC-MS/MS operation

PFAS Library

Oregon State University

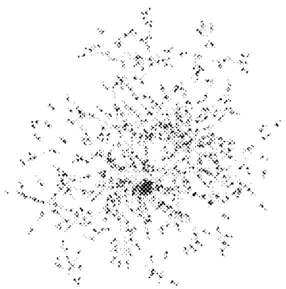
- Collect background information on reference material
  - Source, purity, concentration
- As needed, perform validation of purity and concentration (QToF to screen for additional PFAS not on label)
- Develop quantification strategy if no native/isotope labeled standards
- Library is a unique source of reference materials
  - Opportunity to observe and add PFAS to suspect screening lists
  - Opportunity/challenge to create analytical methods for nonvolatile and volatile PFAS
  - Leverage knowledge for use on EPA landfill project (NC State, lead)

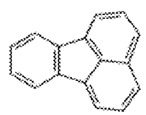
Molecular Dynamics and PBTK: Toxicokinetic Parameterization and Modeling for Diverse PFAS

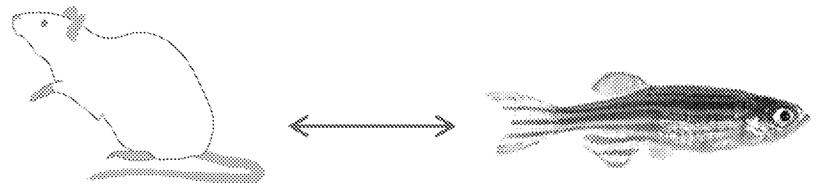
EPA National Priorities: Per- and Polyfluoroalkyl Substances
Grant Kickoff Meeting, Thursday September 5 2019

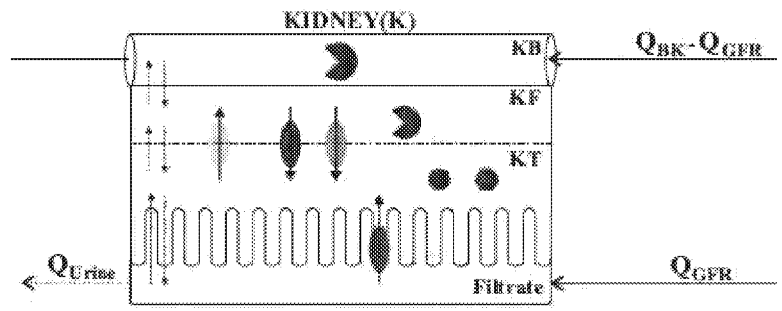
Manoochehr Khazaee and Carla Ng, University of Pittsburgh
Department of Civil & Environmental Engineering

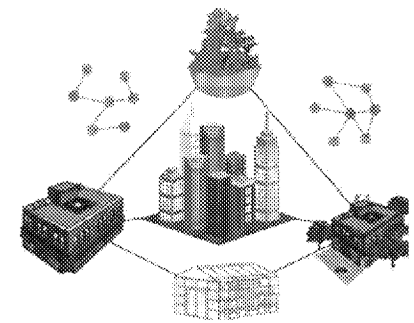
The Ng Lab: multiscale models for insight into chemistry & biology

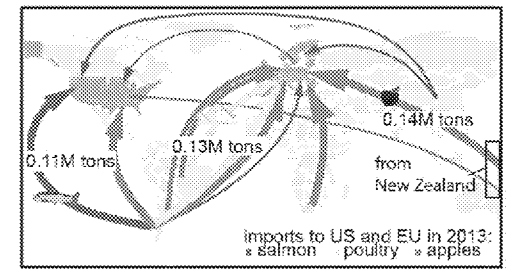




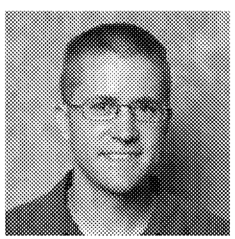








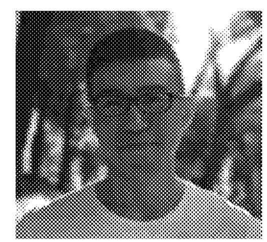
DATA: FOOD TRADE DATA, ORIGIN LABELS



Trevor Sleight

Weixiao Cheng

Manoochehr Khazaee



Zhaokai Dong

Megha Bedi

www.pitt.edu/~carlang

Our role in the project:

Development of new mechanistic pharmacokinetic models for PFAS in both zebrafish and mouse.

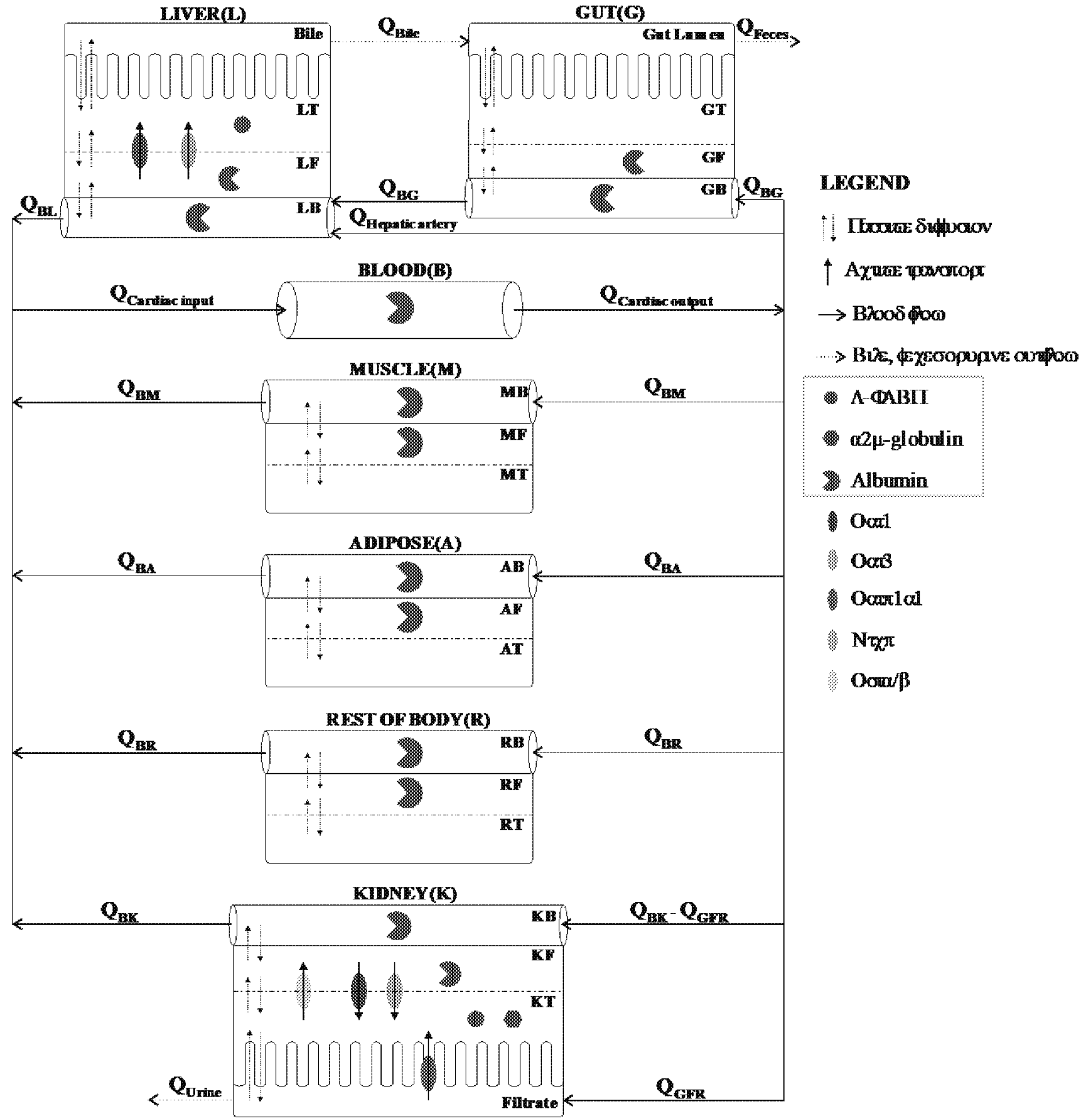
Molecular dynamics simulations to predict PFAS-protein binding and correlate with findings from experimental teams with both tissue distribution and toxic effects.

Our approach:

"Bottom-up PBTK"

<u>Components:</u>
Physiology
In vitro data

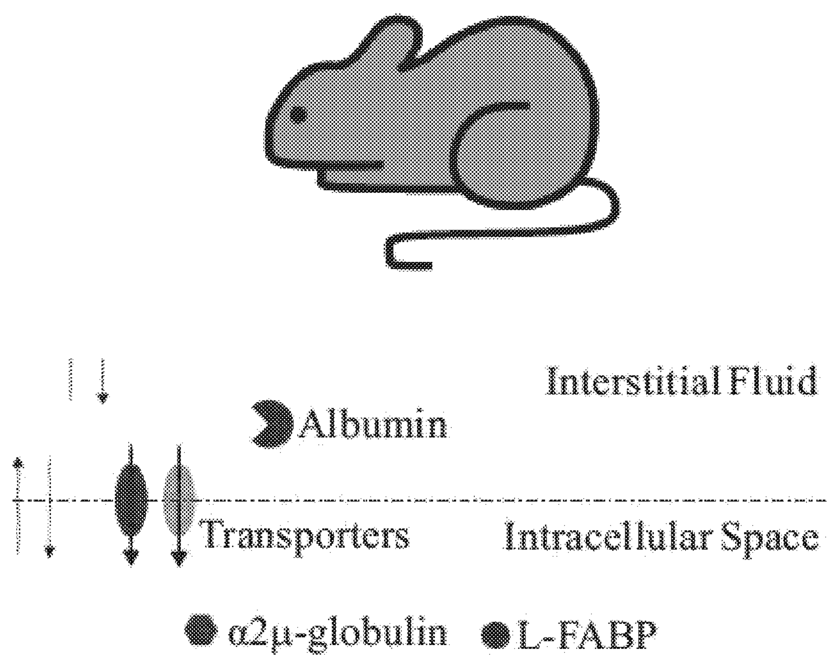
<u>Goal:</u>
No parameters fit to *in vivo* data.
(Test of IVIVE)



Weixiao Cheng

A recent successful case study: PFOA in male rats

72 Independent Parameters



Evaluation Data

7 data sets from 3 studies:

Kemper 2003

Kudo et al. 2007

Kim et al. 2016

High and low doses:

1 mg/kg, 0.1 mg/kg, 0.041 mg/kg

Oral and IV dose

Plasma time course

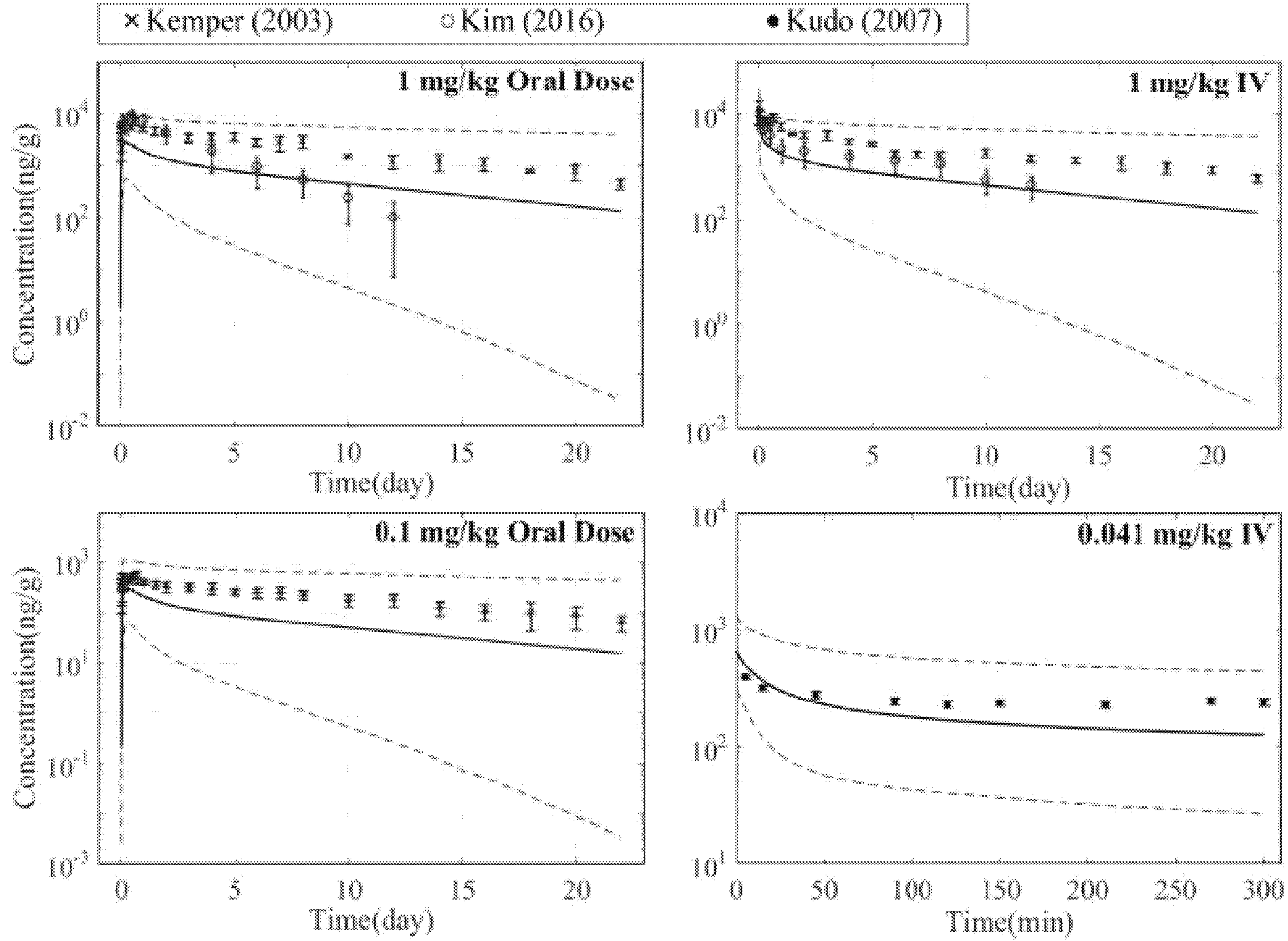
Tissue distribution

Performance

Plasma time course for:

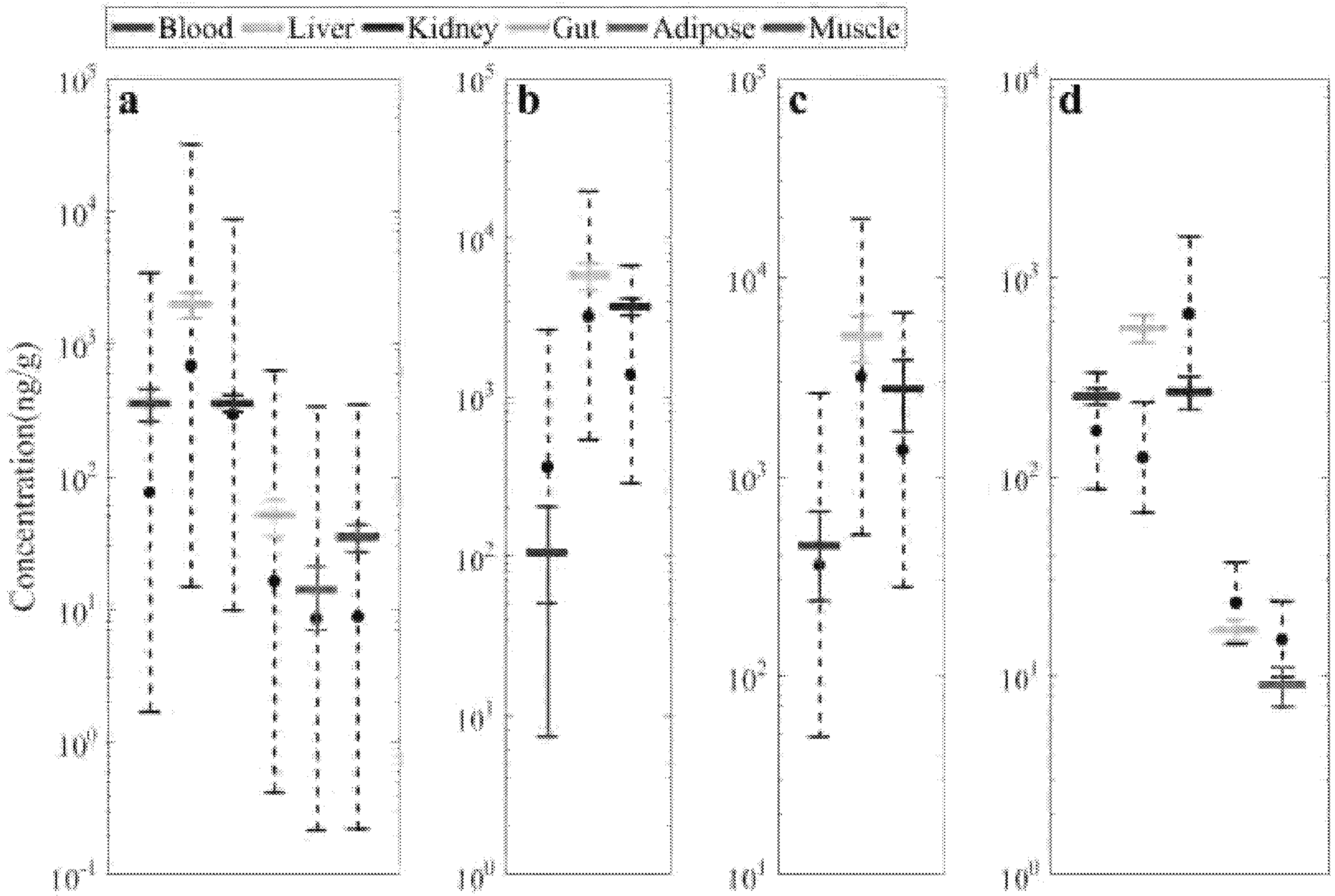
(a) 1mg/kg oral dose
(b) 1mg/kg IV dose

(c) 0.1 mg/kg oral dose
(d) 0.041 mg/kg IV dose.



Cheng & Ng 2017 ES&T

Outcome:
A model that can successfully predict *in vivo* kinetics and distribution based only on *in vitro* chemical-specific data.



(a) 28d after 1mg/kg oral dose (Kemper 2003)

(b,c) 12d after 1mg/kg oral, IV dose (Kim 2016)

(d) **2hr** after 0.041mg/kg IV dose (Kudo 2007)

Cheng & Ng 2017 ES&T

Cheng's work shows the power of a "bottom up" model when sufficient parameterization data are available.

Having reliable mechanistic PBPK models gives us power to evaluate many substances, answer key unknowns.

If we have sufficient information on organism physiology and PFAS interactions with membranes and intracellular proteins.

What happens when appropriate data are lacking?

Environmental Science Processes & Impacts

ROYAL SOCIETY OF CHEMISTRY

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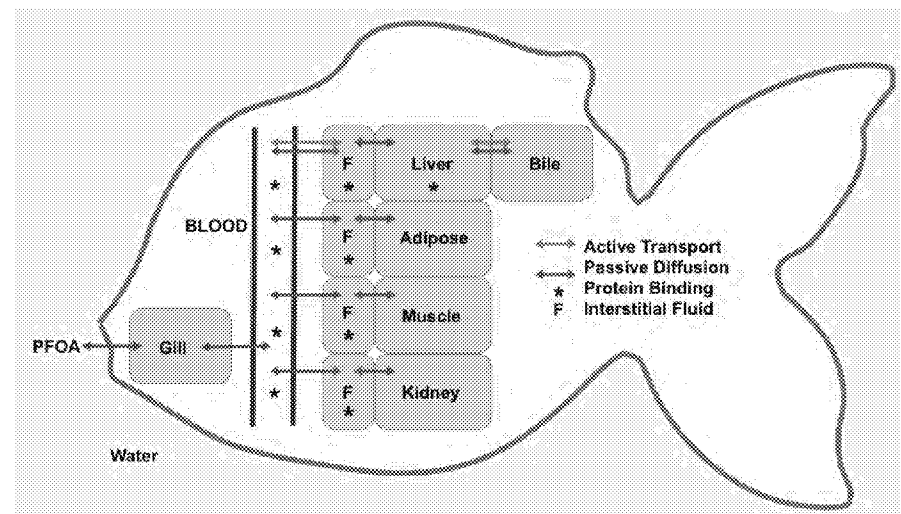
Cite this: DOI: 10.1039/c7em00474e

Evaluating parameter availability for physiologically based pharmacokinetic (PBPK) modeling of perfluorooctanoic acid (PFOA) in zebrafish†

Manoochehr Khazaee and Carla A. Ng *

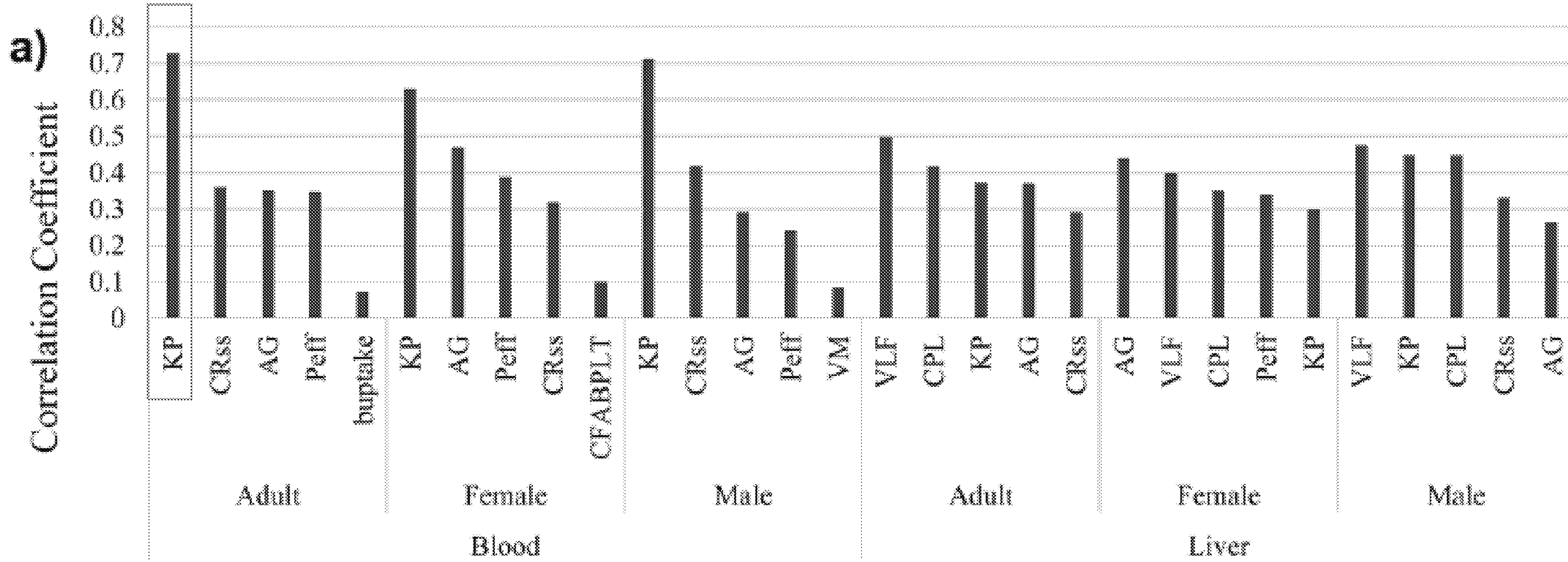


i.	Abb.	References	Species			Method			
						Estimation			
			Zebrafish	Other fish (+1)	Mammalian (+2)	Direct (+1)	Indirect (+1.5)	Experimental	Score
	VL	59	*	—	—	—	—	*	1
	VM	61	*	—	—	*	—	—	2
	VK	62 and 63	*	—	—	—	*	—	2.5
	VA	64	*	—	—	—	—	*	1
	VB	58	*	—	—	—	—	*	1
	VG	59	*	—	—	*	—	—	2
	VLF	41	—	*	—	*	—	—	3
	VKF	41	—	*	—	*	—	—	3
	VAF	41	—	*	—	*	—	—	3
	VMF	41	—	*	—	*	—	—	3
	AL	59	*	—	—	—	*	—	2.5
	AK	62, 63 and 66	*	—	—	—	*	—	2.5
	AM	67	*	—	—	—	*	—	2.5
	AA	68	*	—	—	—	*	—	2.5
	AB	69	—	*	—	—	—	*	2
	AG	4	—	*	—	—	*	—	3.5
	QBi	70	—	*	—	—	—	*	2
	QW	42, 88 and 89	*	—	—	*	—	—	2
	QB	42	*	—	—	*	—	—	2
	QBL	33 and 40	—	*	—	—	—	*	2
	QBK	33 and 40	—	*	—	—	—	*	2
	QBM	33 and 40	—	*	—	—	—	*	2
	QBA	33 and 40	—	*	—	—	—	*	2
	CPB	44	*	—	—	—	—	*	1
	CFABP	71	—	*	—	—	—	*	2
	CPL	72 and 90	—	—	*	—	—	*	3
	CPK	—	—	—	*	—	*	—	4.5
	CPM	74	—	—	*	—	—	*	3
	CPA	74	—	—	*	—	—	*	3
	K_P	45 and 76–78	—	—	*	—	*	—	4.5
	K_{FABP}	79	—	—	*	—	—	*	3
	P_{eff}	28	—	—	*	—	—	*	3
	CR_{SS}^{C-w}	28	—	—	*	—	—	*	3
	b_{clear}	75	—	—	*	—	—	*	3
	b_{uptake}	75	—	—	*	—	—	*	3

Reviewed 96 papers published between 1956 and 2017, including physiological, protein-, and transport-related parameters for 8 species.

Parameter Scoring:

Based on species and method of estimation or measurement (lower is better).



Sensitivity Analysis allows us to prioritize parameters with greatest influence.

Target for experimental and modeling work.

CPB	44	*	—	—	—	—	*	1
CFABP	71	—	*	—	—	—	*	2
CPL	72 and 90	—	—	*	—	—	*	3
CPK	—	—	—	*	—	*	—	4.5
CPM	74	—	—	*	—	—	*	3
CPA	74	—	—	*	—	—	*	3
K_P	45 and 76–78	—	—	*	—	*	—	4.5
K_{FABP}	79	—	—	*	—	—	*	3
P_{eff}	28	—	—	*	—	—	*	3
CR_{SS}^{C-w}	28	—	—	*	—	—	*	3
b_{clear}	75	—	—	*	—	—	*	3
b_{uptake}	75	—	—	*	—	—	*	3

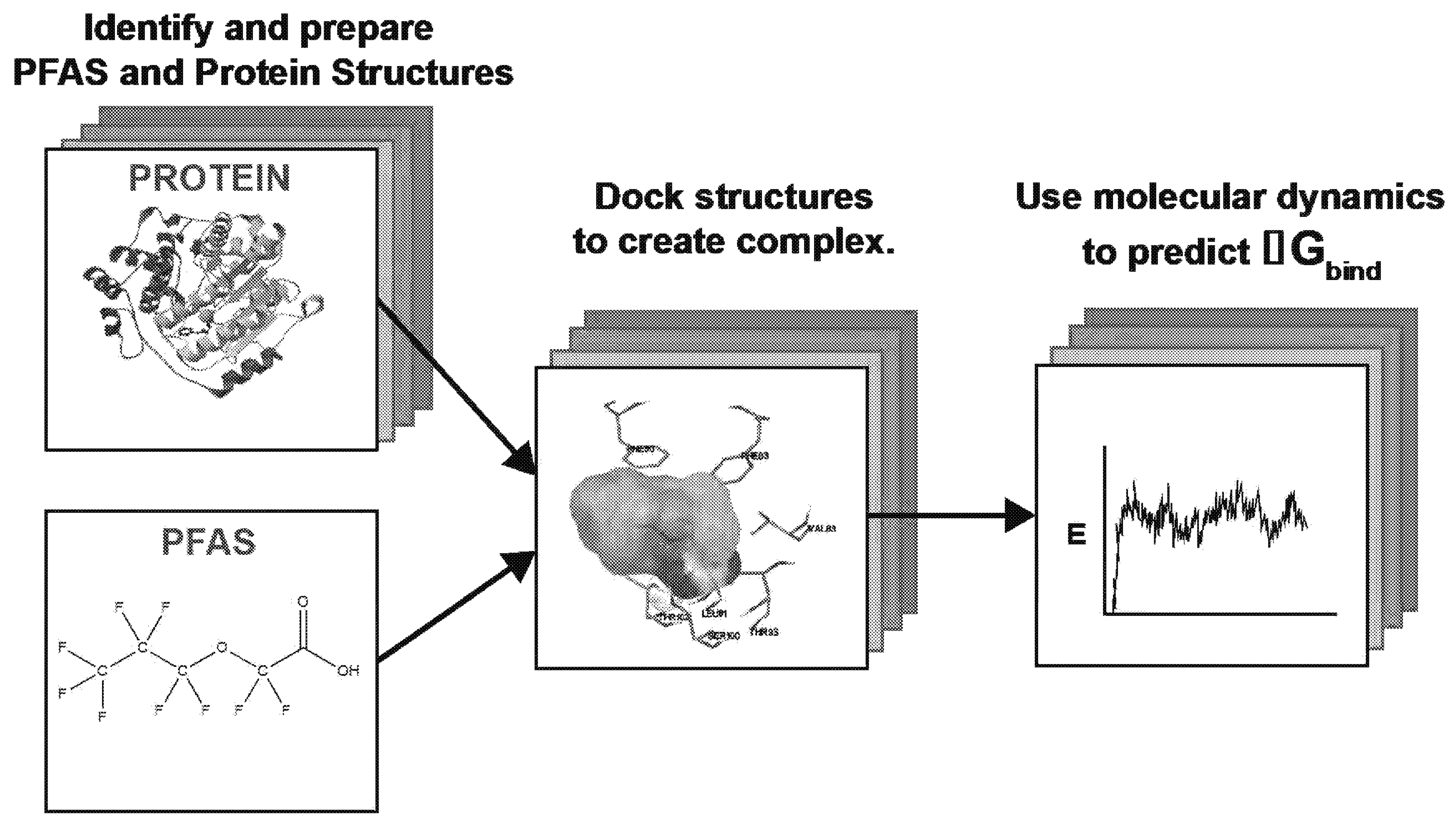
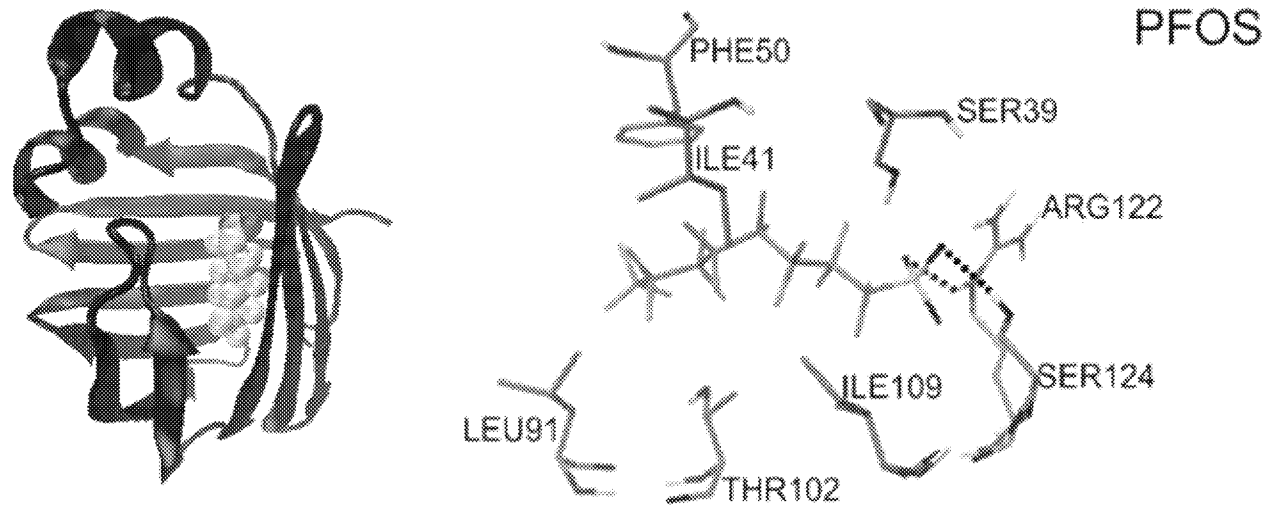


Figure 8: Molecular Dynamics (MD) work ow to predict PFAS-protein interactions.

Modeling Tool:
Molecular Dynamics.

Often used for drug candidate screening, can predict relative binding affinity of protein-ligand complexes.

A molecular dynamics framework for PFAS:

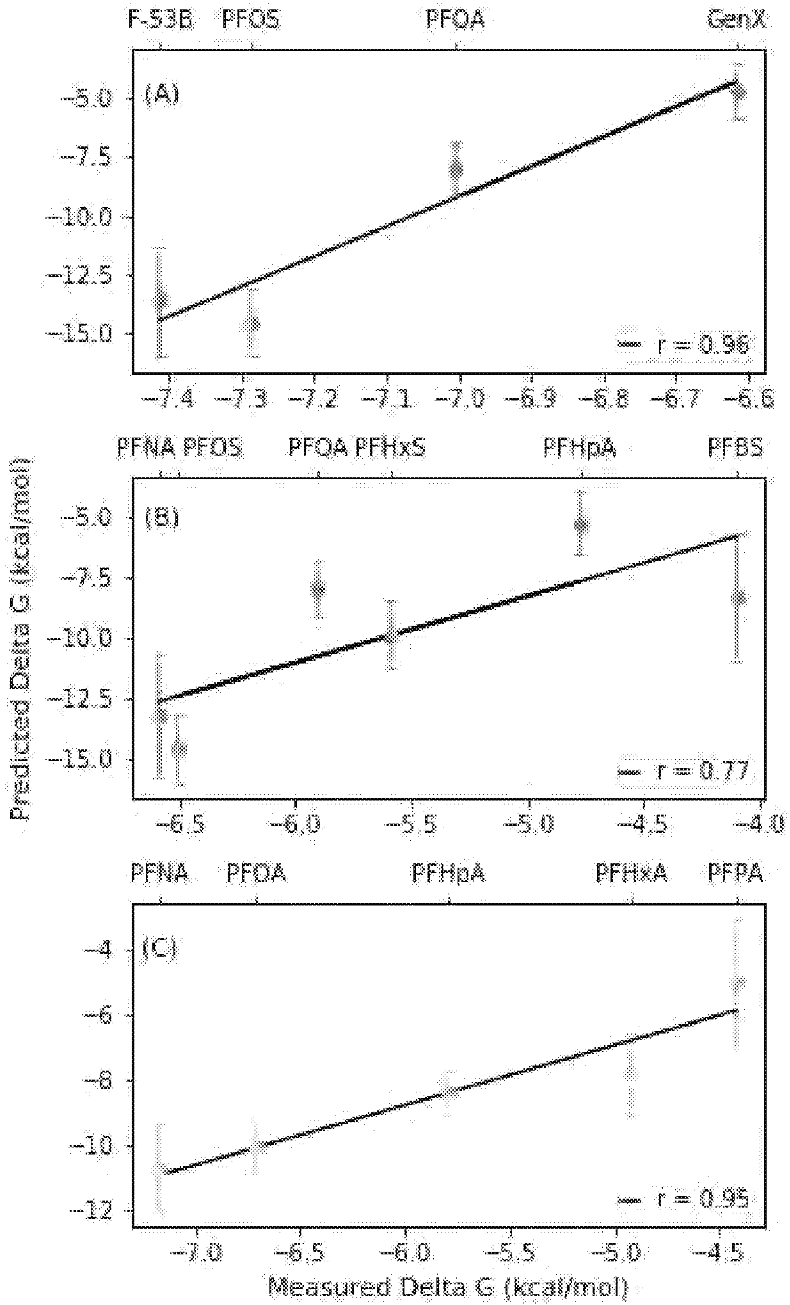


$$\Delta G_{\text{bind}} = G^{\text{complex}} - G^{\text{LFABP}} - G^{\text{PFAS}}$$

$$G = < E_{\text{bond}} + E_{\text{el}} + E_{\text{vdw}} + G_{\text{polar}} + G_{\text{nonp}} - TS >$$

$$\Delta G_{\text{bind}} = RT \ln \frac{K_{\text{d}}}{c_0}$$

Link to bioaccumulation and toxicity



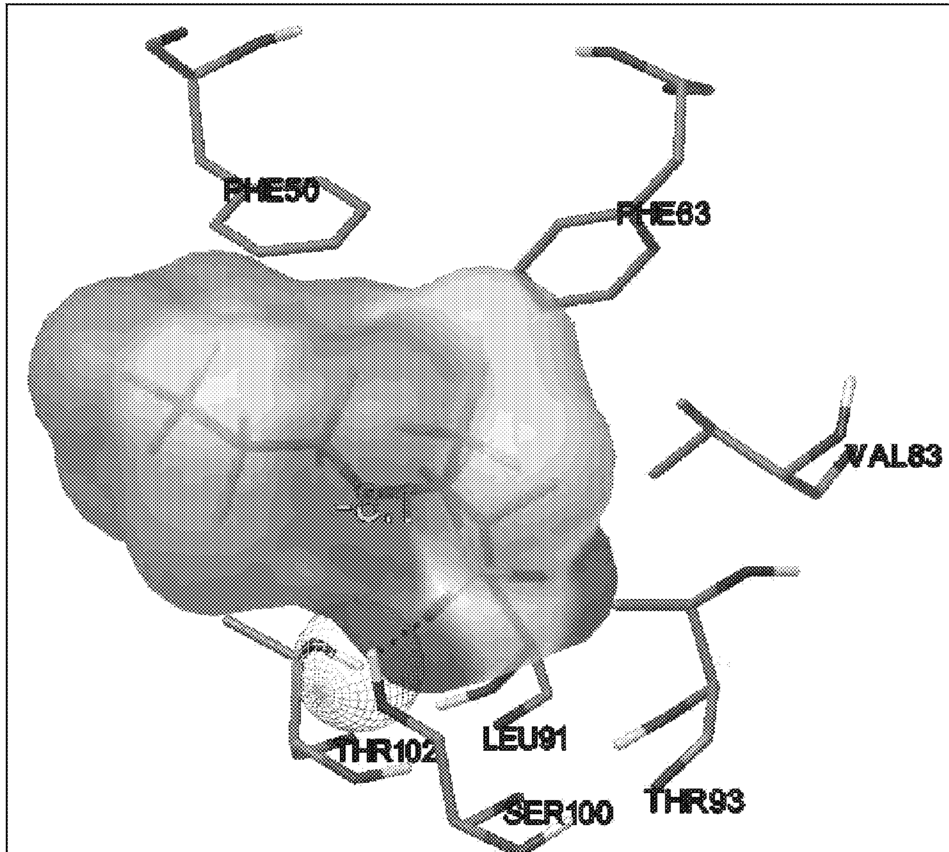
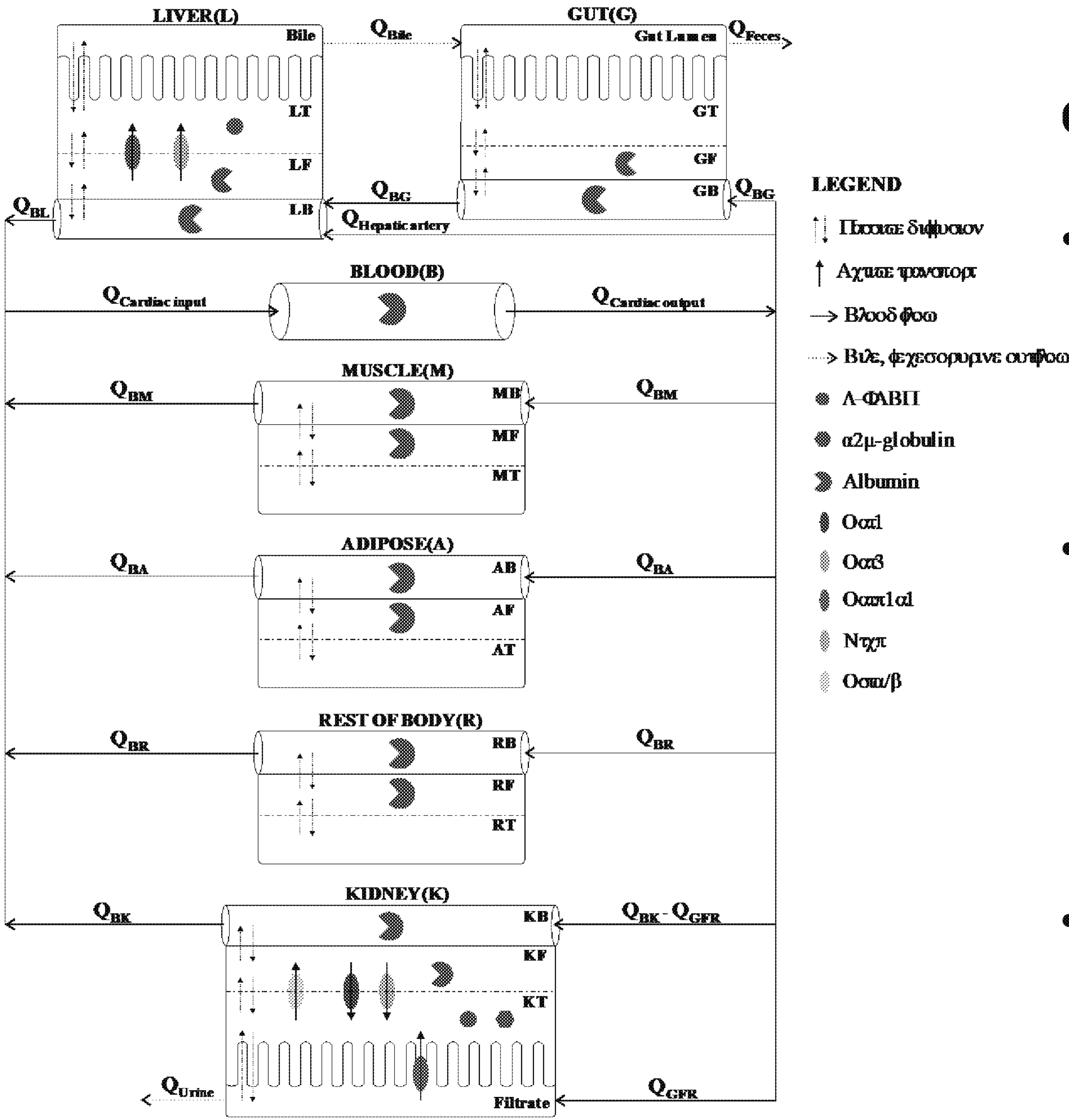


Figure 9: Hydrophobic and electrostatic interactions of GenX with amino acid residues in rat L-FABP

Cheng & Ng 2018 ES&T

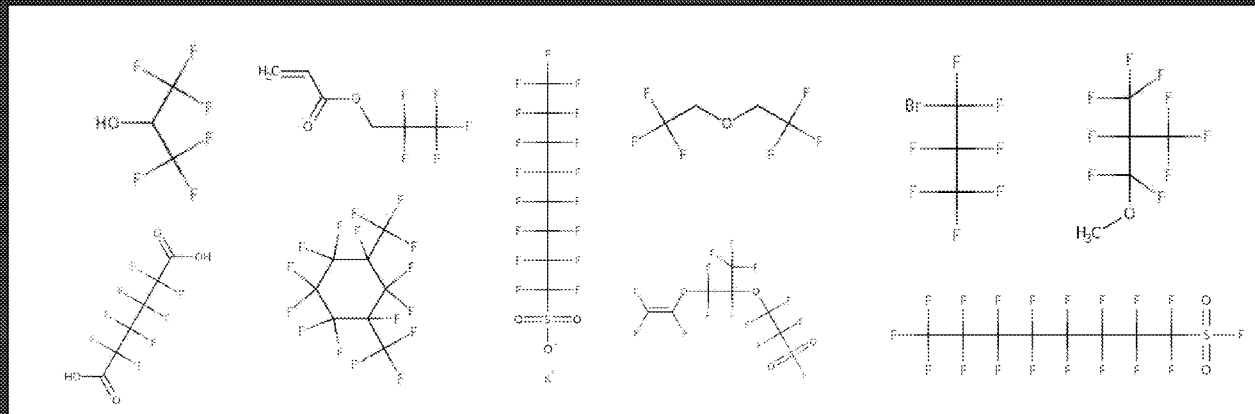
Our modeling workflow can predict the *relative* binding affinities of both legacy and "emerging" PFAS, and give insight into why those interactions occur.



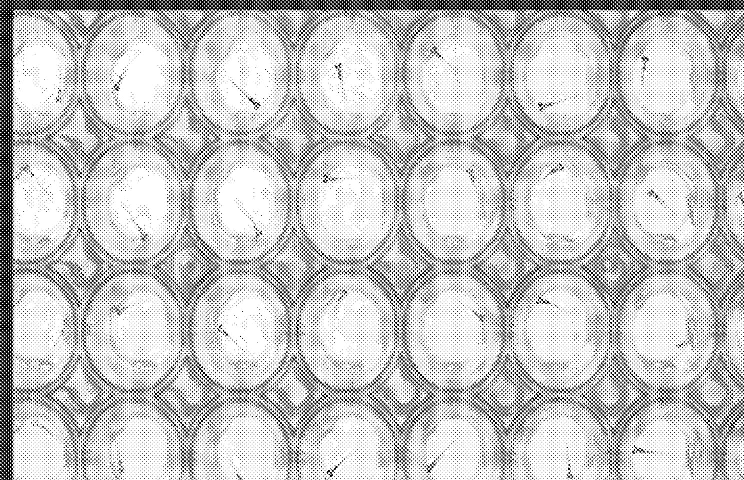
Our key outputs and information goals:

- Based on toxicokinetic modeling and observed tissue distributions, which tissues are important to include/describe (for which PFAS)? (e.g. PFAS-specific sinks)
- Based on molecular dynamics and kinetic observations, which intra- and extracellular proteins and membrane transporters likely control uptake and elimination (thereby biological half-lives)?
- What differences are observed between males and females that can be incorporated in the models? For which PFAS?

EPA
United States
Environmental Protection
Agency

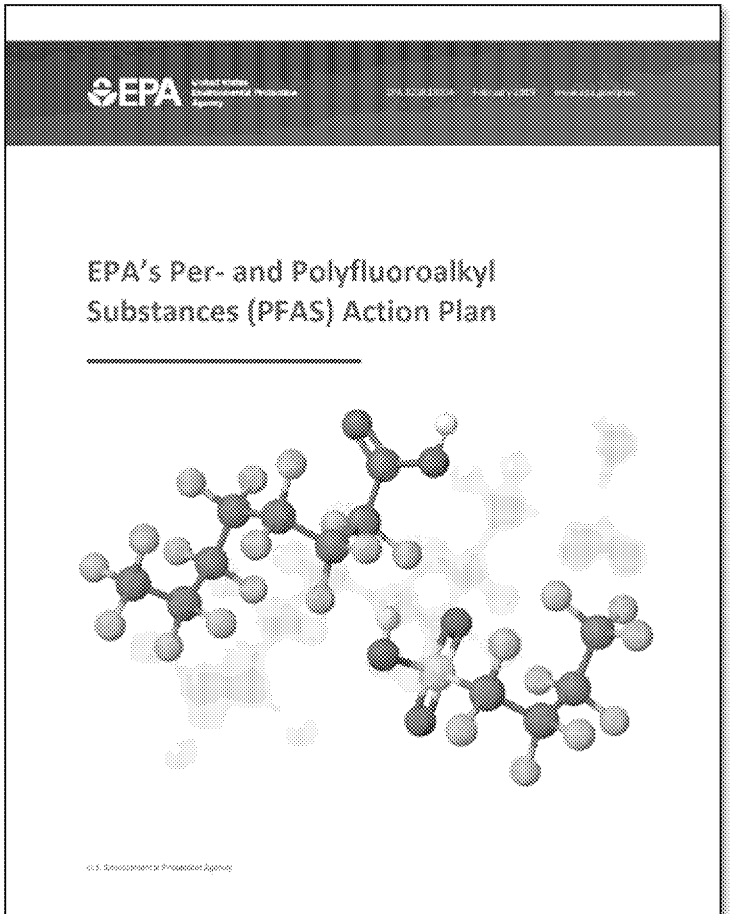


September 5, 2019



Stephanie Padilla
ISTD
U.S. EPA

EPA is Using New Approach Methods to Help Fill Information Gaps

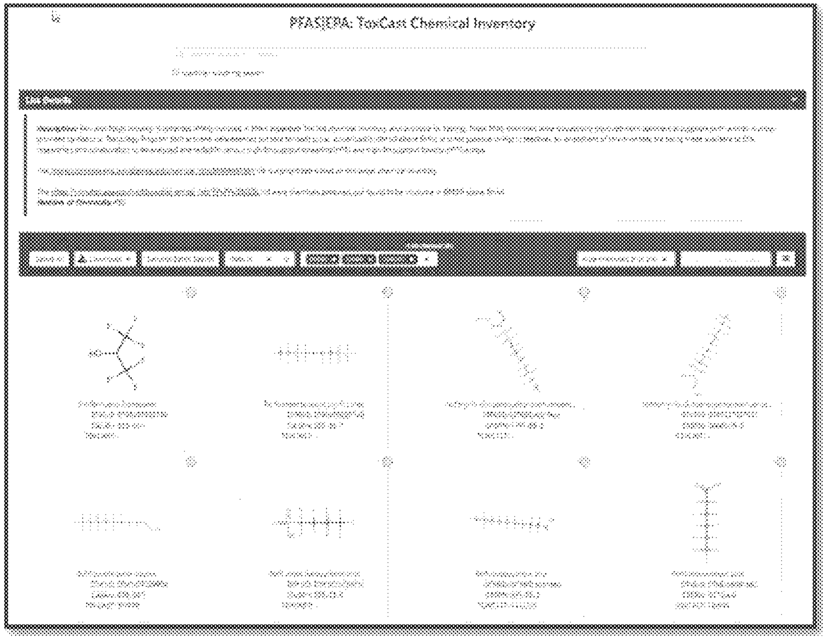


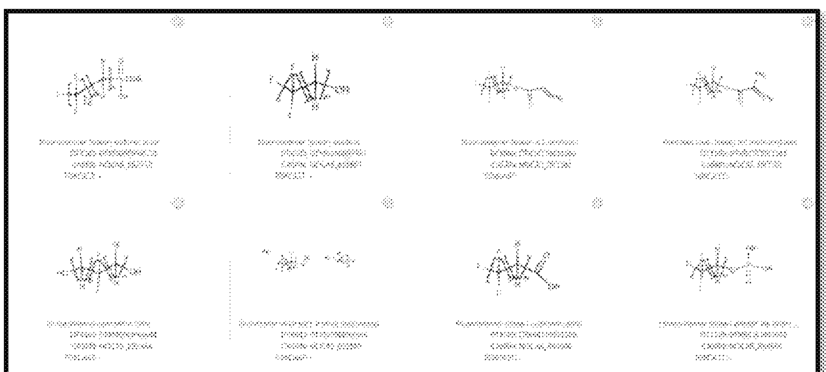
Research Area 1: *What are the human health and ecological effects of exposure to PFAS?*

- Using computational toxicology approaches to fill in gaps. For the many PFAS for which published peer-reviewed data are not currently available, the EPA plans to use new approaches such as high throughput and computational approaches to explore different chemical categories of PFAS, to inform hazard effects characterization, and to promote prioritization of chemicals for further testing. These data will be useful for filling gaps in understanding the toxicity of those PFAS with little to no available data. ***In the near term***, the EPA intends to complete assays for a representative set of 150 PFAS chemicals, load the data into the CompTox Chemicals Dashboard for access, and provide peer-reviewed guidance for stakeholders on the use and application of the information. ***In the long term***, the EPA will continue research on methods for using these data to support risk assessments using New Approach Methods (NAMs) such as read-across and transcriptomics, and to make inferences about the toxicity of PFAS mixtures which commonly occur in real world exposures. The EPA plans to collaborate with NIEHS and universities to lead the science in this area and work with universities, industry, and other government agencies to develop the technology and chemical standards needed to conduct this research.

EPA United States Environmental Protection Agency

Assembled a PFAS Chemical Library for Research and Methods Development





- Attempted to procure ~3,000 based on chemical diversity, Agency priorities, and other considerations
- Obtained 480 total unique chemicals
  - 430/480 soluble in DMSO (90%)
  - 54/75 soluble in water (72%) (incl. only 3 DMSO insolubles)
- Issues with sample stability and volatility
- Categories assigned based on three approaches
  - Buck et al., 2011 categories
  - Markush categories
  - OECD categories
  - Manual assignment

Kathy Coutros, Chris Grulke, and Ann Richard

Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing

EPA United States Environmental Protection Agency


ehp Environmental Health Perspectives

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Brief Communication

Open Access

A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing

Grace Patlewicz, Ann M. Richard, Antony J. Williams, Christopher M. Grulke, Reeder Sams, Jason Lambert, Pamela D. Noyes, Michael J. DeVito, Ronald N. Hines, Mark Strynar, Annette Guiseppi-Elie, and Russell S. Thomas

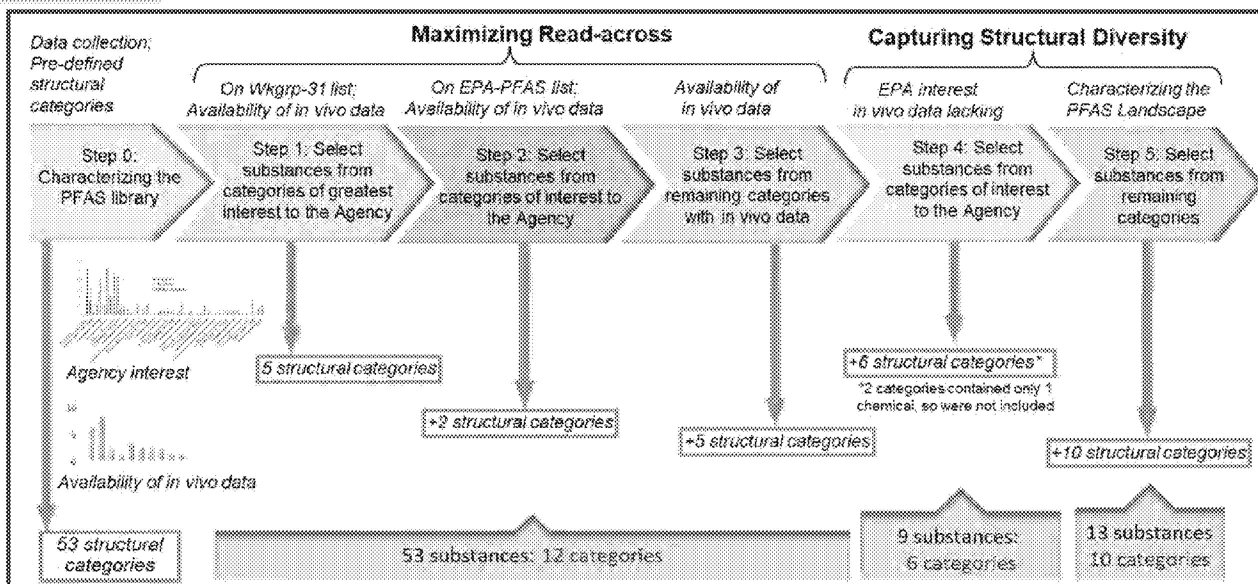
Published: 11 January 2019 | CID: 014501 | https://doi.org/10.1289/EHP4555

Goals:

- Generate data to support development and refinement of categories and read-across evaluation
- Incorporate substances of interest to Agency
- Characterize mechanistic and toxicokinetic properties of the broader PFAS landscape

Selected 150 PFAS in two phases representing 83 different categories

- 9 categories with > 3 members
- Lots of singletons



United States Environmental Protection Agency

In Vitro Toxicity and Toxicokinetic Testing

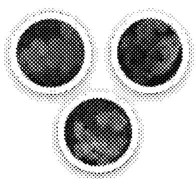
Toxicological Response	Assay	Assay Endpoints	Purpose
Hepatotoxicity	3D HepaRG assay	Cell death and transcriptomics	Measure cell death and changes in important biological pathways
Developmental Toxicity	Zebrafish embryo assay	Lethality, hatching status and structural defects	Assess potential teratogenicity
Immunotoxicity	Bioseek Diversity Plus	Protein biomarkers across multiple primary cell types	Measure potential disease and immune responses
Mitochondrial Toxicity	Mitochondrial membrane potential and respiration (HepaRG)	Mitochondrial membrane potential and oxygen consumption	Measure mitochondrial health and function
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption	ACEA real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
General Toxicity	Attagene cis- and trans- Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in hepatotoxicity
	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
	High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology
Toxicokinetic Parameter	**Assay**	**Assay Endpoints**	**Purpose**
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to plasma protein	Measure amount of free chemical in the blood

*Assays being performed by NTP and EPA

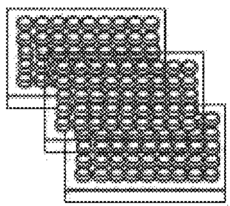
High-Throughput Phenotypic Profiling (aka 'Cellular Pathology')

United States Environmental Protection Agency

Multiple Cell Types

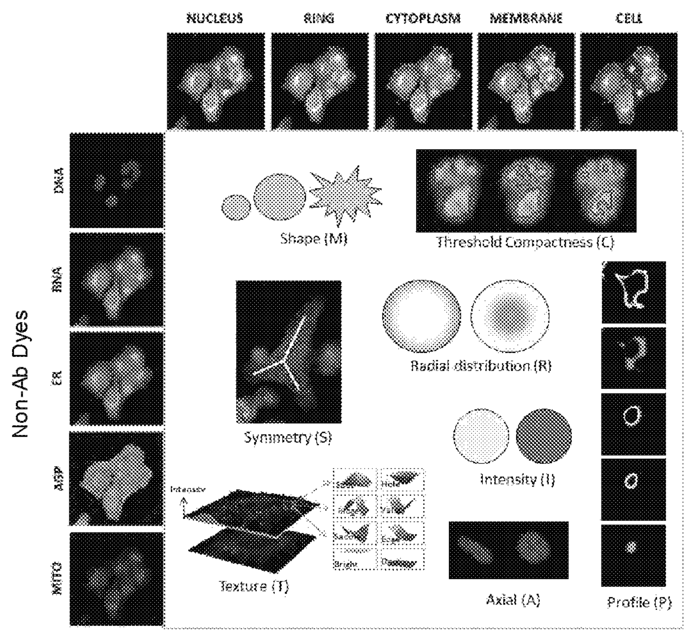


Concentration Response Screening



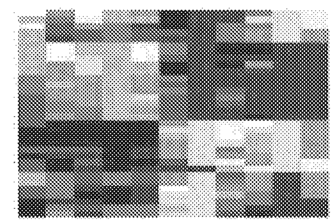
Multi-Parameter Cellular Phenotypic Profiling

Cell Compartments

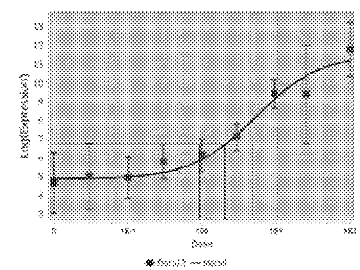


~1,300 endpoints

Mode-of-Action Grouping



Concentration Response Modeling

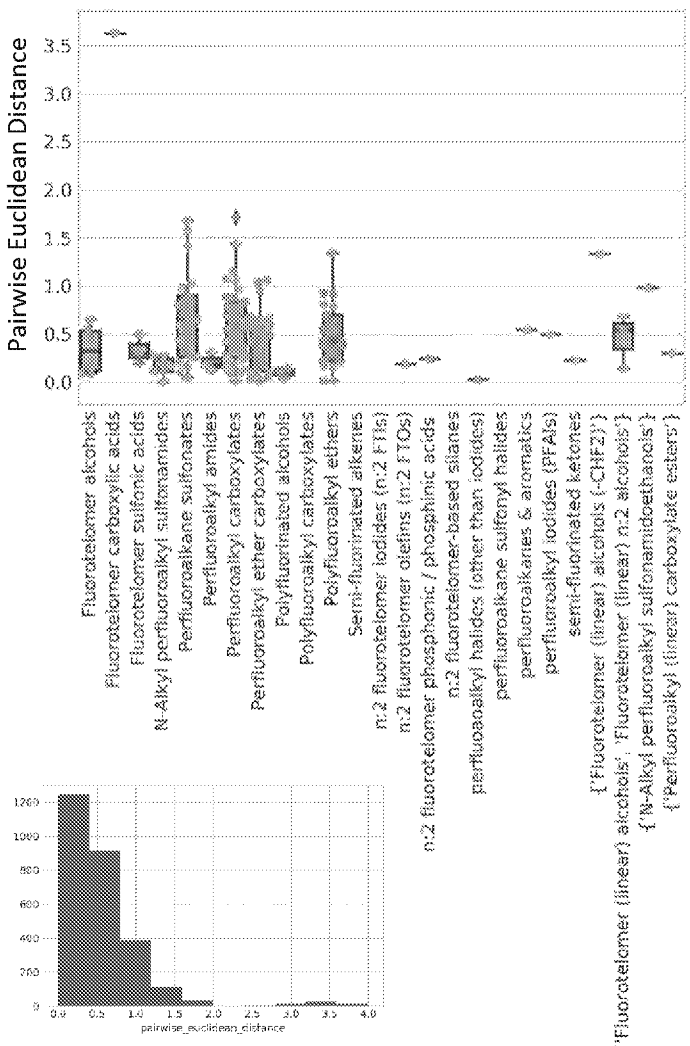


Joshua Harrill and Johanna Nyffeler

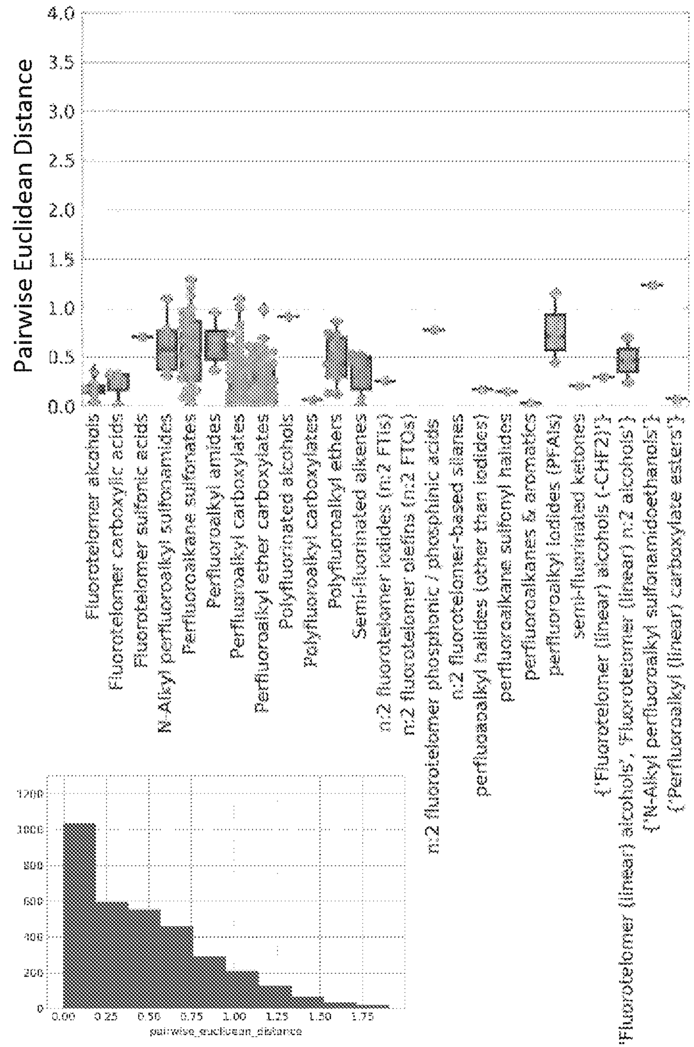
Preliminary Category-Based Analysis of the Phenotypic Profiling Assay

United States Environmental Protection Agency

MCF7 Cells



U2OS Cells

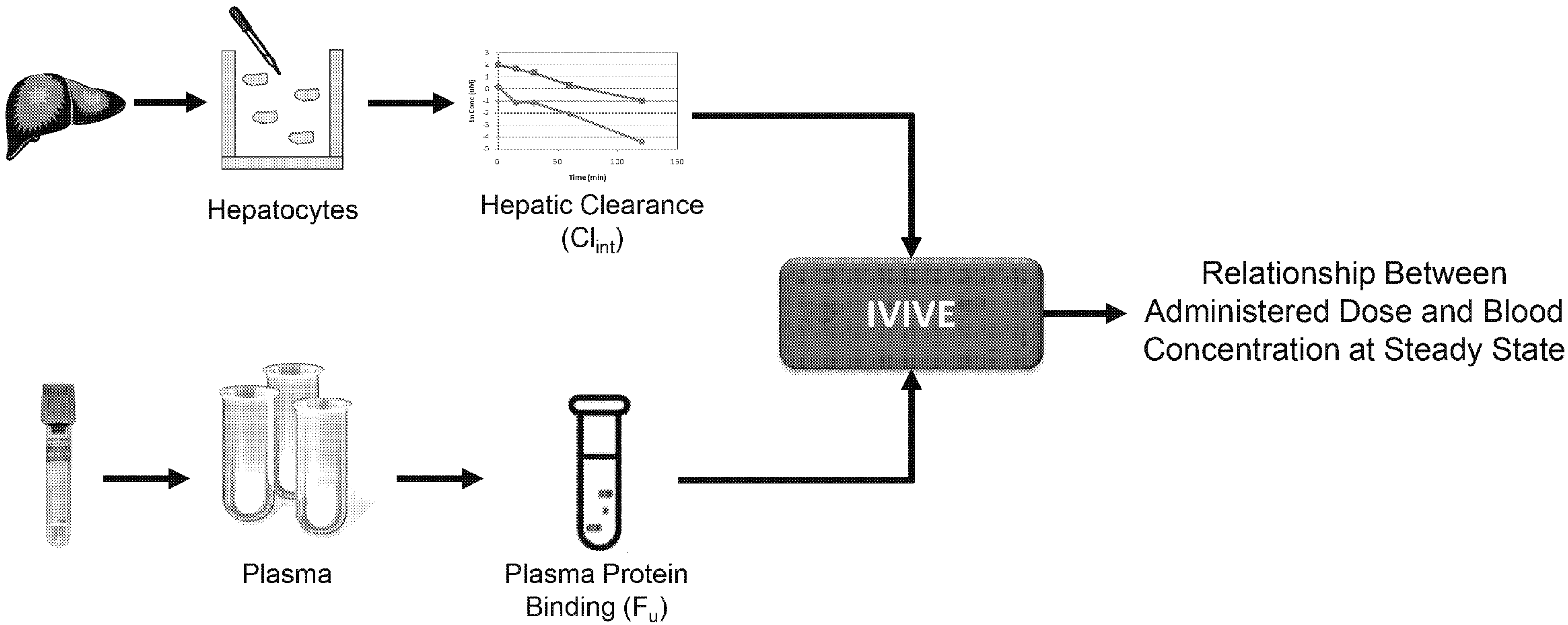


Office of Research and Development

*Results are preliminary. Chemicals still undergoing analytical QC.

Joshua Harrill, Johanna Nyffeler, and Grace Patlewicz

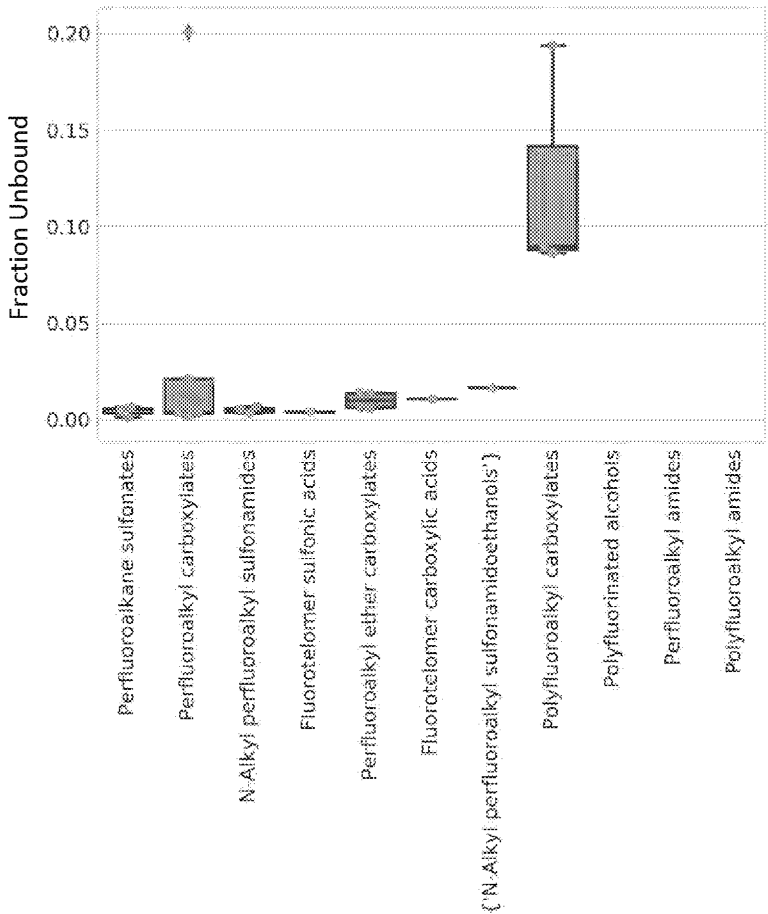
In Vitro Toxicokinetic Assays and *In Vitro*-to-*In Vivo* Extrapolation

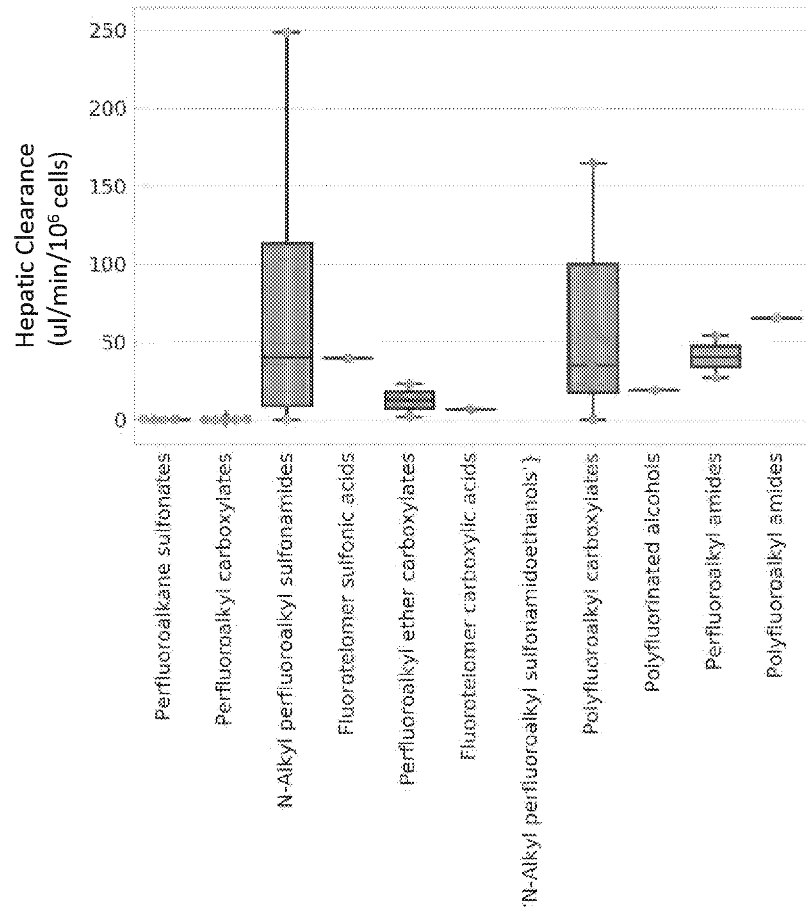


Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

David Crizer, Barbara Wetmore

Preliminary Analysis of the Toxicokinetic Assays





*Results are preliminary. Chemicals still undergoing analytical QC.

David Crizer, Barbara Wetmore, and Grace Patlewicz

Zebrafish: The New Laboratory Rat

Strengths and Weaknesses

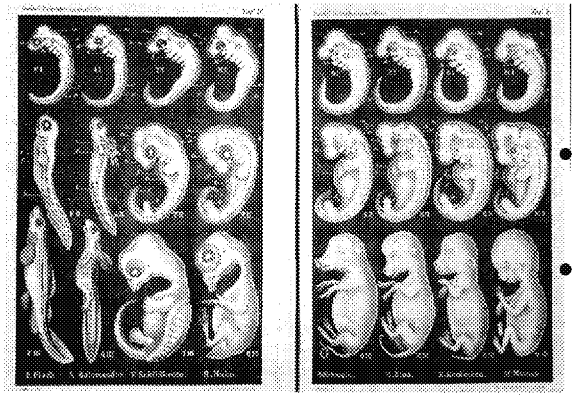
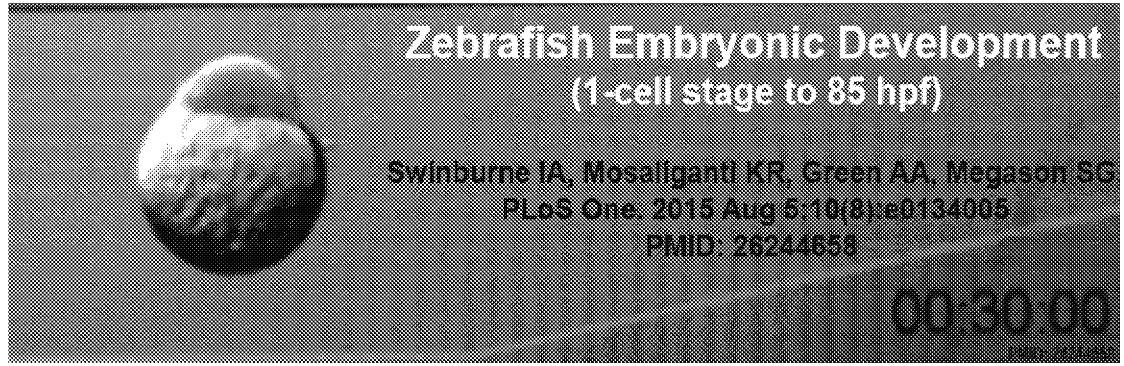


FIG. 41 Comparisons of embryos in three different stages of evolution. Ernst Haeckel, *The Evolution of Man: A Popular Exposition of the Principal Points of Human Ontogeny and Phylogeny* (1883).



- **All vertebrates develop using the same design.**
- **Therefore we can use "lower" level vertebrates to screen for toxicity in humans.**

- Rapid development (organogenesis is complete within 4 days) (see movie above)
- Transparent embryo
- Developmental pathways are homologous with other vertebrates
- Easy to manipulate genome
- Translational model serving both human- and eco-toxicology
- Apical endpoints, including functional assessments
- Metabolic capability
- Tested over 150 PFAS so far

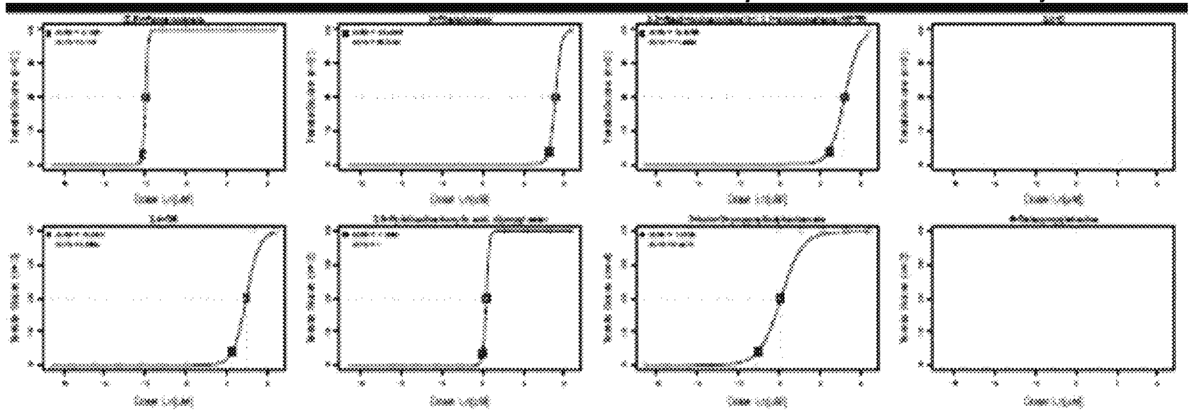
Concerns:

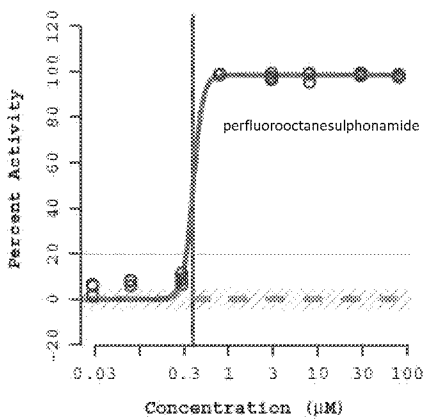
- difficult to assign mechanism without further tests
- knowing the internal dosage of chemical is not simple

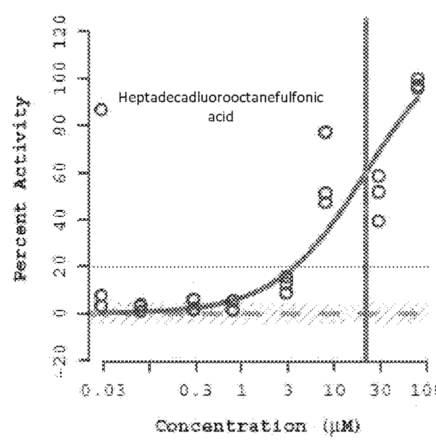
Zebrafish Research

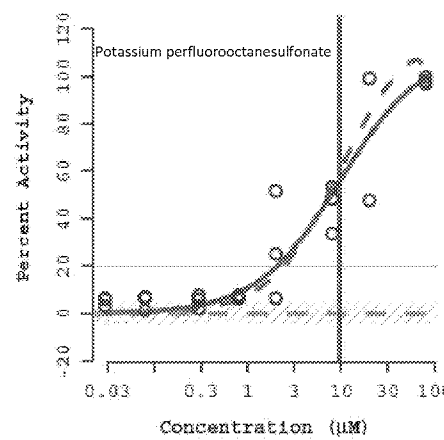
Use zebrafish larvae as a medium throughput screening tool to reduce uncertainties in risk assessment

Evaluating methods to screen and prioritize large numbers of chemicals for developmental toxicity

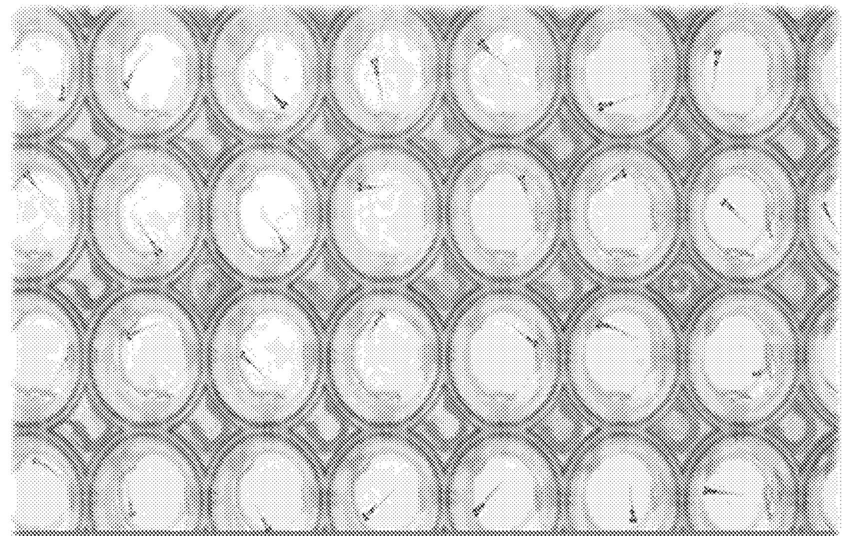








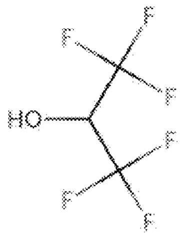
EXPERIMENTS AND RESULTS:

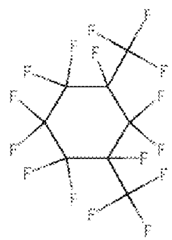


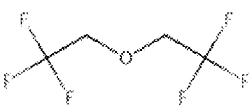
- Three plates each on two days (n=6);
- 8 chemicals per plate
  - Highest dose was 100 µM
- Positive control on each plate
  - (this is important as there are so many negatives)
- All experimental conditions on each plate
- Blinded to chemical treatment
- Plates are dosed and immediately sealed to prevent evaporation of potentially volatile chemicals
- Transferred to vehicle at 5 days of age
- Assessed for various endpoints on 6 days of age
- Only about <20% hits, and usually at only the highest dose. Hit rate may be confounded by stability and volatility.
- We will be retesting all 150 chemicals.

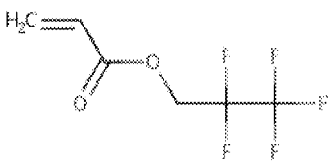
David Korest, Neha Menon, Deborah Hunter, Kimberly Jarema

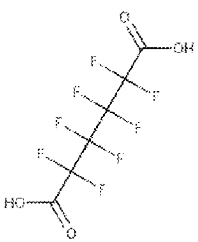
Take Home Messages…

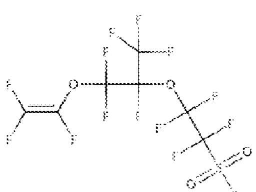












- Chemical curation efforts are important to harmonize structure, naming, and identifiers across the PFAS space
- A chemical library of 430 PFAS has been assembled for chemical screening, analytical method development, and other research needs
- A subset of 150 PFAS selected for *in vitro* toxicity and toxicokinetic testing to refine/support read across categories and to estimate a conservative point-of-departure
- *In vitro* toxicity and toxicokinetic testing and analysis are underway and demonstrate the diverse biological activities and toxicokinetic properties of PFAS